The effect of dietary protein source on the metabolism and performance of ewes in late pregnancy and early lactation

Thesis

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THE EFFECT OF DIETARY PROTEIN SOURCE ON THE METABOLISM AND PERFORMANCE OF EWES IN LATE PREGNANCY AND EARLY LACTATION

BY

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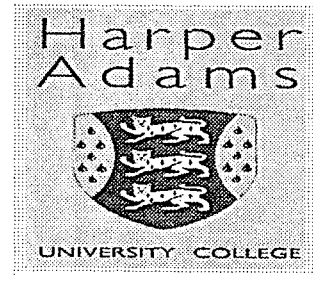

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David Handford

July 2001

ABSTRACT

Three experiments were carried out to investigate the effects of dietary protein source on the metabolism and performance of straw fed, twin-bearing ewes in late pregnancy and early lactation. In the first experiment, 72 ewes were fed concentrate diets which contained either fishmeal or formaldehyde treated soya-bean meal. The concentrate was either fed alone, or at a reduced rate with feedblocks *ad libitum* in a two by two factorial design. Ewes fed fishmeal lost more condition after parturition and had lambs which grew quicker than ewes fed treated soya-bean meal ($P<0.05$). Ewes offered feedblocks produced colostrum with a higher immunoglobulin G (IgG) concentration at birth, had lambs with a higher litter weight than those fed concentrate alone ($P<0.05$). In the second experiment, 60 ewes were fed concentrates containing either untreated or formaldehyde treated rapeseed meal or field beans in a two by two factorial design with an additional control diet containing fishmeal. Ewes fed fishmeal had a higher *pre partum* condition score gain and had lambs which grew slower with a lower litter weight at 28 days than ewes fed all other diets. Ewes fed rapeseed meal had a higher total colostrum yield at birth than ewes fed field beans ($P<0.05$). Ewes fed formaldehyde treated protein sources had a reduced yield of total milk, protein, solids not fat and lactose at 21 days *post partum* than ewes fed untreated protein sources ($P<0.05$). In the third experiment, 44 ewes were fed concentrate diets containing either fishmeal, untreated soya-bean meal, formaldehyde treated soya-bean meal or formaldehyde treated soya-bean meal with added rumen protected methionine. Ewes fed formaldehyde treated soya-bean meal with added methionine produced colostrum between 12 and 16 hours *post partum* with a higher fat concentration and a higher yield of fat and crude protein than ewes fed fishmeal ($P<0.05$). Ewes fed formaldehyde treated soya-bean meal with added methionine had lambs which grew quicker and were heavier at 28 days of age than those fed fishmeal ($P<0.05$).

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'Man does not live by words alone, despite the fact that sometimes he has to eat them'

Adlai Stevenson

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ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BHB	β-hydroxybutyrate
BSA	Bovine serum albumin
CP	Crude protein
CS	Condition score
CTAB	Cetyle trimethylammonium bromide
DMI	Dry matter intake
DUP	Digestible undegradable protein
EDTA	Disodium ethylene diamine tetra-acetate dihydrate
EE	Ether extract
ERDP	Effective rumen degradable protein
FME	Fermentable metabolisable protein
Ig	Immunoglobulin
MCP	Microbial crude protein
ME	Metabolisable energy
MP	Metabolisable protein
N	Nitrogen
NAN	Non ammonia nitrogen
NDF	Neutral detergent fibre
NEFA	Non esterified fatty acids
OM	Organic matter
PMSG	Pregnant mare serum gonadotrophin
RID	Radial immunodiffusion
SNF	Solids not fat
TCA	Tricarboxcylic acid
UDP	Undegradable dietary protein
VFA	Volatile fatty acid

CHAPTER 1. REVIEW OF THE LITERATURE

1.1 INTRODUCTION

There are currently around 20 million breeding ewes in the United Kingdom producing 402 000 tonnes of sheep meat which is worth approximately £586 million per annum to the UK agricultural industry (MLC, 2000). The main contributing factor to the financial superiority of the top third of producers is higher lamb sales which accounted for 83% of the increase in financial output per ewe for the top third compared with the bottom third producers in lowland spring lambing flocks in 1999 (Table 1.1).

Table 1.1 *Financial results (£/ewe) compiled from 88 lowland spring lambing flocks selling most of their lambs off grass in 1999*

Financial results (£/ewe)	Bottom third producers	Top third producers	Superiority (Top third - bottom third)
Output			
Lamb sales and valuations	45.52	59.87	+14.35
Wool	1.26	1.47	+0.21
Ewe premium subsidy	13.19	14.58	+1.39
Gross receipts	**56.97**	**75.92**	**18.95**
Purchased lambs	0.00	0.06	-0.06
Flock replacement cost	8.45	6.45	+2.00
Total output	**48.52**	**69.41**	**20.89**

(adapted from MLC, 2000).

Nutrition during late pregnancy, through its effects on lamb birth weight and synthesis of colostrum, has profound effects on early lamb mortality (Rattray, 1992) whilst nutrition during lactation influences both milk yield and subsequent lamb growth. Ewes may have an increased requirement for protein, which does not break down in the rumen, both during late pregnancy

(due to the large weight gain of the foetus, colostrum synthesis and the reduction in maternal food intake) and during early lactation (ewes rearing twin lambs need to produce high milk yields and consequently often mobilise body fat as a result; Robinson, 1983a). Fishmeal is a suitable protein source for this situation, providing high levels of undegradable protein (UDP) with a high relative value (Sheehan and Hanrahan, 1989). However, more recently, concerns have been expressed over the effects of industrial fishing (House of Lords, 1996) and of the effects of animal proteins in ruminant diets (GAFTA, 1997). The use of vegetable proteins as an alternative to fishmeal has disadvantages. Vegetable proteins supply a lower proportion of rumen undegradable protein compared to fishmeal and lack in the supply of certain essential amino acids.

The aim of the current series of experiments was to investigate the potential of vegetable protein sources as alternatives to fishmeal in diets for pregnant and lactating ewes.

1.2 PROTEIN DIGESTION IN RUMINANTS

1.2.1 Introduction

Ruminants depend for their amino acid supply on the mixture of food protein which escapes ruminal degradation and on the microbial protein which is formed as the result of rumen fermentation (Wallace, 1994). The main factors affecting the supply of amino acids to the small intestine of the ruminant animal are the amount and type of energy and protein yielding substrates fed and the physiological state of the animal (AFRC, 1993).

1.2.2 Conversion of dietary protein to ammonia in the rumen

1.2.2.1 Proteolysis

Proteolysis is a property shared by all the main categories of rumen microorganisms (Cotta and Hespell, 1986; Wallace and Cotta, 1988 and Wallace, 1996a). Bacteria are most important in the breakdown of soluble proteins and probably protein in general (Broderick *et al.*, 1991). Most bacterial protease activity is associated with the cell surface of bacteria (Wallace, 1996a) and therefore the first step of proteolysis is the adsorption between the cell surface layers of bacteria and the protein (Nugent and Mangan, 1981; Wallace, 1985). Proteases arising from mixed bacterial populations are predominantly cysteine proteases, with smaller proportions of serine and metalloproteases (Brock *et al.*, 1982; Kopecny and Wallace, 1982). A schematic representation of the process of protein degradation is given in Figure 1.1.

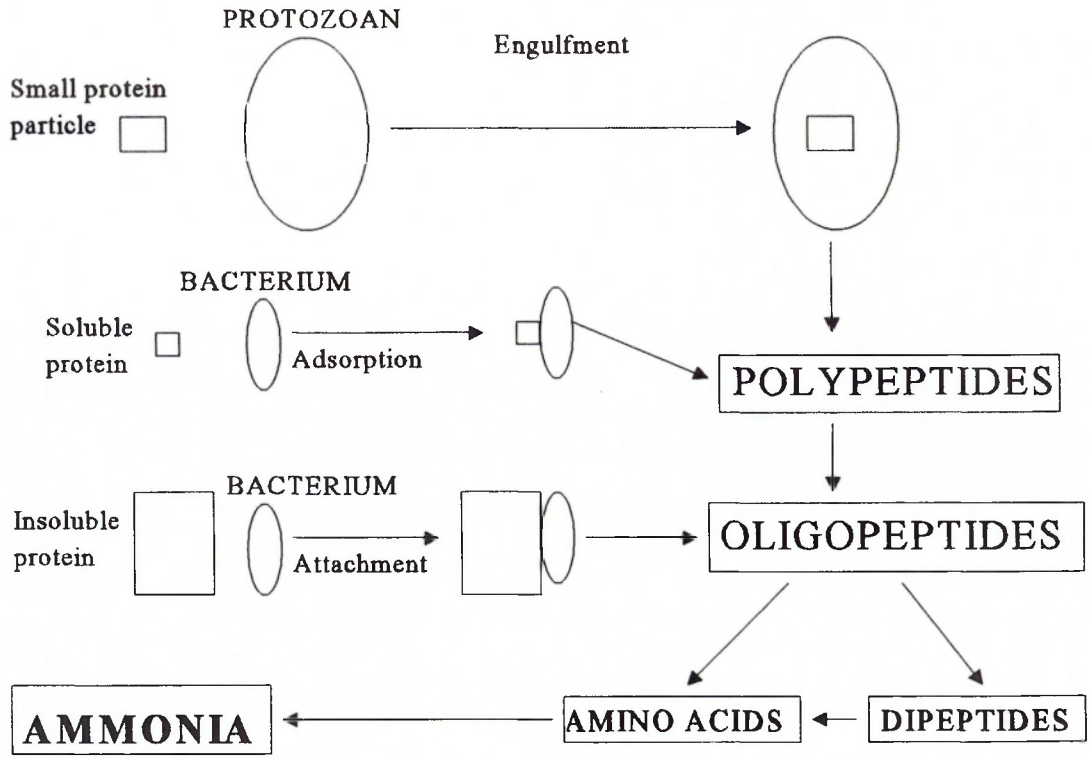

Figure 1.1 Pathway of degradation of intact protein to ammonia by microorganisms in the rumen (Broderick *et al.*, 1991).

The proteolytic activity of the rumen fluid, the proportion of proteolytic species and the predominant proteolytic species present all appear to be influenced by diet (Wallace, 1996a). Many different bacterial species are involved in the degradation of protein (Wallace and Brammall, 1985) and up to 50% of bacteria isolated from the rumen may be proteolytic (Wallace, 1996a). The predominant species of proteolytic bacterium found in the rumen of most animals is *Prevotella ruminicola* (Blackburn and Hobson, 1962; Fulghum and Moore, 1963; Hazlewood and Nugent, 1978 and Wallace and Brammall, 1985). Feeding fresh herbage appears to increase the proportion of proteolytic species present, with activities several times higher than those found with dry rations (Hazlewood *et al.*, 1983 and Nugent *et al.*, 1983), whilst cereal diets can give higher activities than dry forage diets (Siddons and Paradine, 1981). Different types of supplemental protein may also give rise to different activities (Hazlewood *et al.*, 1983 and Wallace *et al.*, 1987). In addition to the diet affecting the total numbers of

proteolytic species present, diet can also influence the proportions of individual species. Hazlewood *et al.* (1983) found that cows fed a hay / concentrate ration had a high proportion of *Butyrivibrio fibrisolvens* (50% of proteolytic isolates) and *Streptococcus bovis* (44% of proteolytic isolates), whilst the predominant proteolytic isolate in cows fed freshly cut alfalfa was *Streptococcus bovis.* Other species of proteolytic rumen bacteria include *Selenomonas ruminantium, Ruminobacter amylophilus, Eubacterium ruminantium, Fusobacterium* sp and *Clostridium* sp (Morrison and Mackie, 1996).

Cilate protozoa and anaerobic fungi also carry out proteolysis, peptidolysis and deamination but to a lesser extent than bacteria (Brock *et al.*, 1982 and Kopecny and Wallace, 1982). Several species of protozoa including *Ophryoscolex* sp, *Entodinium caudatum* and *Eudiplodinium medium* (Williams *et al.*, 1961 and Naga and el-Shazly, 1968) have been shown to have proteolytic activity. Cilate rumen protozoa exhibit a variety of protease activity, the most important of which being cysteine and aspartic proteases (Forsberg *et al.*,1984).

1.2.2.2 Peptidolysis

Some peptides and amino acids may resist degradation for a sufficient period of time to resist ruminal degradation (Chen *et al.*, 1987) and loss of peptides and amino acids can occur across the rumen wall (Leibholz, 1971; Webb *et al.*, 1992). However, the main fate of peptides and amino acids arising from proteolysis is to be taken up by the microorganisms and then to be incorporated into protein or broken down to ammonia (Wallace, 1996b). Different peptides are broken down at different rates and it appears that it is the structure of the N-terminus that is crucial in determining the rate of breakdown (Wallace, 1997). If glycine or proline is at or next to the N-terminus, or if the peptide has a net negative charge, then the rate of peptide degradation tends to be slow (Wallace, 1997).

The predominant type of peptide hydrolysis in sheep and cattle by mixed rumen microorganisms is dipeptidyl peptidase, where dipeptides are cleaved sequentially from the N-terminus of peptides (Figure 1.2; Wallace and McKain, 1989; Wallace, 1996b). *Prevotella ruminicola* is the only rumen microbial species with significant dipeptidyl peptidase activity (Wallace and McKain, 1991). However, activity is present in other rumen species including cilate protozoa (Wallace, 1994).

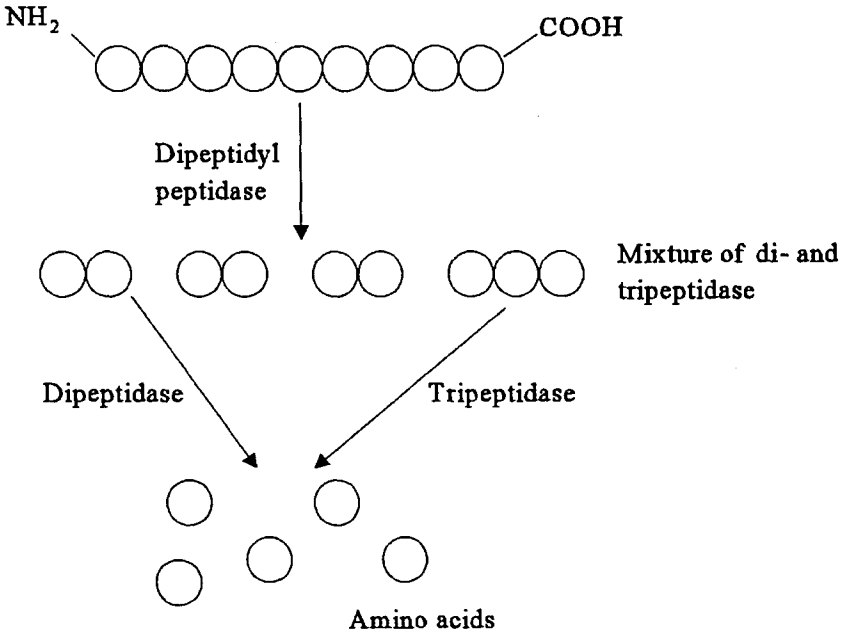

Figure 1.2 Biphasic breakdown of peptides by rumen bacteria and by *P. Ruminicola* (Wallace 1997).

1.2.2.3 Amino acid degradation

The main fate of amino acids in the rumen is degradation into ammonia (Wallace, 1996b). However, substantial quantities are incorporated directly into microbial protein (Nolan, 1975; Leng and Nolan, 1984). Amino acids are the main source of ammonia in the rumen (Al-Rabbat *et al.*,1971; Mathison and Milligan, 1971 and Chalupa, 1976). Different amino acids are broken down at different rates and into different products (Wallace, 1994). Recent research has concentrated on the microbial population which is primarily responsible for the production of

ammonia from amino acids. It appears that there are two distinct groups of bacteria that are responsible for deamination in the rumen. The first group are low in numbers, but have a specific activity for ammonia production that is an order of magnitude greater than that of other species (Chen and Russell, 1988; Russell *et al.*, 1988; Chen and Russell, 1989 and Russell *et al.*, 1991). The species isolated were *Clostridium sticlandii, Clostridium aminophilum* and *Peptostreptococcus anaerobius* (Paster *et al.*, 1993), none of which fermented sugars, but used amino acids as their main source of carbon and energy as well as nitrogen. Other bacteria, which are higher in numbers, but with much lower specific activity for ammonia production have been isolated from the rumen (Yang and Russell, 1993). The species of bacteria isolated are *Butyrivibrio fibrisolvens, Megasphaera elsdenii, Prevotella ruminicola, Selenomonas ruminantium* and *Streptococcus bovis.* Unlike the previously listed bacteria, most of these species ferment sugars (Wallace, 1996b).

1.2.3 Microbial protein synthesis

1.2.3.1 The requirement for ammonia, pre-formed amino acids and peptides for microbial growth

Studies on the nutritional requirements of rumen bacteria have shown that ammonia is a major nitrogen source for microbial growth. Ammonia is derived from either the microbial catabolism of amino acids and peptides or from the action of bacterial urease on urea of dietary or blood origin. Values for the total amount of microbial-N derived from ammonia can range from 18 to 100% (Atasoglu *et al.*, 1999). It is generally accepted that ammonia is an important source of nitrogen for bacterial growth under most feeding situations (Nolan, 1975; Aharoni *et al.* 1991) and is essential for the growth of several species of rumen bacteria (Allison, 1969 and 1970 and Bryant, 1974). It is possible for the rumen microbial population to grow with no pre-formed amino acids at all (Virtanen, 1966), although it appears that the yield of microbial

protein may not be optimal under these conditions (Hume, 1970; Maeng and Baldwin, 1976). Virtanen (1969) found that cows given diets containing protein-free carbohydrates, supplemented with urea and ammonium salts could produce an upper limit of 4000 litres of milk / year, much lower than could be expected on similar diets containing protein. However, diets that supply pre-formed amino acids and peptides can increase the efficiency of microbial protein synthesis compared to those which supply only ammonia (Chen *et al.*, 1987; Chen and Russell, 1988). Many studies have shown that diets which contain some pre-formed amino acids and peptides do support a higher rate of fermentation and microbial growth yield (Cruz Soto *et al.*, 1993; Chikunya *et al.*, 1996), and this has been attributed to the effects of pre-formed amino acids and peptides on non-cellulolytic rumen bacteria (Russell *et al.*, 1992; Chikunya *et al.*, 1996).

Studies based on pure cultures have shown that cellulolytic bacteria use ammonia as their main source of nitrogen, while non-cellulolytic species use a higher proportion of pre-formed peptides and amino acids (Wallace *et al.*, 1999). These assumptions are used in the Cornell system for modelling rumen fermentation (Russell *et al.*, 1992; Alderman, 2001). However, this may be an over-simplification. The results of Atasoglu *et al.* (1999) demonstrated that the proportion of microbial cell-N formed from ammonia in a mixed microbial population is not fixed but varies with the proportions of NH_3-N and total N available for growth. These proportions of NH_3-N and total N available for growth will vary according to diet and, for meal fed animals, according to the time of day (Wallace and McKian, 1990). The predominantly non-cellulolytic species of rumen bacteria, *Prevotella bryantii, Selenomonas ruminantium and Streptococcus bovis* use peptides and amino acids, when in abundance, for between 95 and 99% of their protein synthesis (Wallace, 1999). At concentrations of peptides similar to that found in the rumen (1g of peptides / litre) 68, 87 and 46% of bacterial amino acid-N were

derived from ammonia by *Prevotella bryantii, Selenomonas ruminantium and Streptococcus bovis* respectively (Wallace, 1999). It appears that the dietary variation in *de novo* incorporation of NH_3 reported by mixed rumen microorganisms (e.g. Atasoglu *et al.*, 1999) reflects the heterogeneity of the microbial population. Therefore, different dietary regimes will support different proportions of bacterial species, each with a different capacity for *de novo* amino acid synthesis and each with differing abilities to use alternative nitrogen (N) sources when dietary conditions change (Wallace, 1999).

1.2.4 Microbial growth yield

Microbes ferment both structural and non structural plant carbohydrates (Van Houtert, 1993). The volatile fatty acids (VFA) produced, whilst being a waste product to the microbes, are absorbed and utilised by the host animal (Van Houtert, 1993). The adenosine triphosphate (ATP) produced during microbial fermentation is used by the microbes for maintenance and for the production of new cells. Bauchop and Elsden (1960) defined the microbial yield as Y_{ATP}, which is expressed as the quantity of bacterial dry matter (g) synthesised from one mole of ATP and assumed a constant value of 10.5. However, Southamer and Bettenhausen (1973) demonstrated that values could range from 4.6 to 20.9 and were affected by both energy and protein source. It is, however, common practice to express microbial efficiency as g of N per kilogram of organic matter (OM) digested in the rumen (Wallace, 1994) and the average value for microbial growth yield recommended by ARC (1984) is 32g N per kilogram of OM digested in the rumen.

1.2.4.1 The influence of rumen degradable protein, fermentable energy and rumen outflow rate on microbial growth yield

In order to produce rumen conditions which allow maximal microbial growth, both rumen

degradable nitrogen and rumen fermentable energy supply must be adequate and the ratio of effective rumen degradable nitrogen : fermentable energy must be optimal (ERDP:FME; AFRC, 1993). In situations where N supply is not adequate, uncoupled fermentation may occur, resulting in substrate catabolism without microbial growth (Wallace, 1994). Conversely, if energy supply to the rumen is limiting, this may limit the efficient utilisation of N (Stern, 1986). ARC (1984) expressed the production of microbial protein (g) as a single linear relationship of 7.3 g of microbial protein per mega joule of metabolisable energy (ME; ARC, 1984) but this made no allowance for the effects of feeding level on rumen outflow rate.

At lower rumen outflow rates the bacterial residence time in the rumen increases and bacterial requirements for maintenance energy as a proportion of total energy requirement increases (Southamer and Bettenhausen, 1973). Harrison and McAllan (1980) determined that the proportion of ATP used to maintain the rumen flora decreased from 0.65 to 0.37 when the rumen outflow rate increased from 0.02 to 0.083 h^{-1}, whilst Isaacson *et al.* (1975) reported that Y_{ATP} increased from 7.5 to 16.7 g of bacterial DM / mole of ATP when outflow rates were increased from 0.02 to 0.12 h^{-1}. The main factor that affects rumen outflow rate is the level of feeding and more recently AFRC (1993) used the level of feeding in the prediction of microbial protein yield. AFRC (1993) predicted yields of 9, 10 and 11g of microbial crude protein (MCP) per mega joule of FME for ruminants at maintenance (rumen outflow (r) 0.02 h^{-1}), twice maintenance (0.05 h^{-1}) and three times maintenance (0.08 h^{-1}) respectively. AFRC (1993) further modified this approach to avoid the discontinuity of the step approach to rumen outflow rate using the following equation:

$$\text{Yield of MCP/MJ of FME (g)} = 7 + 6(1 - e^{(-0.35L)})$$

Where L = level of feeding (ME intake / maintenance requirement).

However, other factors, such as carbohydrate source, the extent of cell lysis and the degree of protozoal predatation will, in addition to the rumen outflow rate, affect the yield of MCP per MJ of FME (Wallace, 1994).

1.2.5 Rumen degradable and undegradable protein

1.2.5.1 Rumen degradability of protein sources

The effective rumen degradation of dietary protein and hence the amount of N available for the rumen microbes is related to the magnitude of the immediately soluble fraction (*a*) and the rate of degradation (*c*) of the potentially degradable fraction (*b*) (AFRC, 1993). The rumen degradability of dietary protein will also affect the supply of rumen undegradable protein to the small intestine. It can be seen from the data of Witt *et al.* (1999a; Table 1.2) that rumen nitrogen degradability differs depending on source of protein, with those of animal origin tending to be more resistant to degradation than those of plant origin. Samples of the same feed type can differ in ruminal protein degradability depending on source and could be a reflection of previous processing treatment. The ruminal protein degradability of fishmeal is affected by both the predominant species of fish present and the length of storage prior to feeding (Mehrez *et al.*, 1980).

Table 1.2 *The readily soluble N fraction (a), the rate of N degradation (c) of the potentially degradable N fraction (b) and the effective N degradability at fractional rumen outflow rates (r) of 0.05 and 0.08 per hour for winter beans, two sources of rapeseed meal and two sources of fishmeal*

				Effective N degradability:-	
	a	*b*	*c*	r =0.05	r=0.08
Winter beans	0.79	0.22	0.058	0.91	0.88
Rapeseed meal A	0.19	0.58	0.107	0.59	0.52
Rapeseed meal B	0.17	0.59	0.102	0.57	0.50
Fishmeal A	0.30	0.67	0.012	0.43	0.39
Fishmeal B	0.46	0.54	0.013	0.57	0.54

(adapted from Witt *et al.*, 1999a)

1.2.5.2 Influence of rumen outflow rate on the DUP supply to the small intestine

Rumen degradability and hence the supply of rumen undegradable protein is not solely a function of the dietary ingredients, but is affected by factors which affect outflow rate from the rumen as well (Ørskov *et al.*, 1983). Ørskov *et al.* (1983) demonstrated that any differences in rumen degradability due to dietary factors would be less apparent at high outflow rates. In the study of Sheehan and Hanrahan (1989) the effective N degradability (%) of fishmeal was 23.0 to 19.1, whilst soya-bean meal was 68.9 to 28.7 at outflow rates of 0.01/h and 0.1/h respectively. AFRC (1993) used rumen outflow rates of 0.05 and 0.08/h for pregnancy and lactation respectively.

1.2.5.3 Quality of protein reaching the small intestine

Amino acids are supplied to the duodenum of ruminants by microbial protein synthesised in the rumen, undegraded dietary protein and endogenous protein (Stern *et al.*, 1994). Clark *et al.* (1992) in a review of the literature reported that, on average, 59% of non ammonia nitrogen passing the duodenum was of microbial origin, but this figure can vary from 50 - 90% (Bondi, 1981). Microbial activity in the rumen can transform feeds that differ widely in their amino acid composition to a protein mixture in which the amino acid composition is fairly constant (Bergen *et al.*, 1968) and it would appear from data presented by Wallace (1994) that the amino acid composition of bacterial protein is superior to that in many common ruminant feedstuffs. The relative value of protein reaching the small intestine would therefore be dependent on the extent to which the dietary protein resisted degradation in the rumen and on the amino acid composition of the microbial and undegraded protein as well as flow of microbial and undegraded protein from the rumen (Wallace, 1994). A further complication arises due to not all proteins arriving in the small intestine being equally digested, and thus the amino acid availability for absorption is not necessarily the same as the amino acid flow in the

intestinal tract (Schingoethe, 1996). Heat damaged proteins treated to bypass the rumen may not be as digestible as other proteins in the small intestine and in particular lysine availability is often reduced (Schingoethe, 1996).

1.3 NUTRITION DURING LATE PREGNANCY

Since the gain in the mass of the foetus in the last 8, 4 and 2 weeks of gestation is equivalent to 85, 50 and 25% of its birthweight, it is reasonable to expect a relationship between plane of nutrition in late pregnancy and lamb birthweight (Robinson, 1983a). Russel *et al.* (1967b and 1977) and McClelland and Forbes (1969) reported that the degree of undernourishment was negatively related to lamb birthweight. However, other authors, for example McClelland and Forbes (1973) found no such relationship.

Maternal nutrition, as well as influencing lamb birth weight, also affects foetal energy reserves, colostrum production and composition, thus playing a key role in the survival of the neonate (Mellor and Murray, 1985b; Robinson, 1990b; Rattray, 1992). Lambs with higher birth weights are less susceptible to hypothermia and heat stress because they have a lower surface area to volume ratio, higher summit metabolism per unit surface area, higher energy reserves of body lipid and glycogen along with higher amounts of brown adipose tissue (BAT), than smaller lambs from undernourished ewes (Rattray, 1992). Lambs born to under nourished ewes are also more likely to suffer from increased heat losses due to the influences of maternal nutrition on the birth coat (Black, 1983). Also, small weak lambs have less desire to suckle than their larger counterparts. In addition, the poor maternal behaviour exhibited by malnourished ewes leads to increases in lamb mortality (Alexander, 1986).

1.3.1 Energy requirements

The gravid uterus has a requirement for glucose which rises rapidly towards the end of pregnancy (Russel *et al.*, 1967a; Robinson *et al.*, 1977) and the maternal ability to supply this glucose is a key factor regulating foetal growth (Symonds and Clarke, 1996). Reduced blood flow and utero-placental glucose uptake occurs after undernutrition and this is accompanied

by foetal hypoglycaemia (Robinson, 1990b). Despite this, the dry matter intake of ewes is often depressed in late pregnancy, particularly in ewes with large litter sizes (Everts, 1990). This reduction in food intake may occur either because the increase in uterine contents reduces the ability of the rumen to distend, or because the influence of other metabolic factors that diminish appetite increase at this time (Forbes, 1970). In such circumstances the ewe may become carbohydrate deficient (Russel *et al.*, 1967b) and will begin to mobilize body fat reserves. During severe cases of carbohydrate deficiency the amount of mobilised adipose tissue becomes excessive and in such circumstances the lack of available glucose reduces the production of oxaloacetate, a key metabolite in the Tricarboxylic acid (TCA) cycle (Payne, 1989). This, in turn, inhibits the use of the TCA cycle for the oxidation of mobilised fatty acids leading to a build up of ketones (Payne,1989).

1.3.1.1 Effects on lamb birth weight

Nutritional regimens which cause undernutrition through a failure to increase nutrient intake in line with the increasing needs of the rapidly growing foetus influence lamb birth weight through a gradual slowing down of prenatal growth (Mellor and Murray, 1981 and 1982a). Severe and sudden restrictions in food intake, which can often be found during deteriorating weather conditions in hill and upland flocks in the last third of pregnancy will result in a reduction in pre-natal growth of 30-40%, or in some cases a complete cessation of growth (Mellor and Matheson, 1979). Mellor and Matheson (1979) also showed that if severe restriction in nutrient intake is short (up to one week), then partial increases in growth will occur subsequently if nutrient intake is increased. This is not the case for longer periods of undernutrition. Mellor and Murray (1982b) showed that if a reduction in nutrient intake sufficient to cause a 50% reduction in pre-feeding plasma glucose concentrations lasted for 16 days, then foetuses lacked the ability to return to normal growth rates when nutrient intakes

were increased. Russel *et al.* (1967a) reported that levels of nutrient intake designed to be similar to those commonly found in hill flocks reduced lamb birth weights by 10 and 25% in single and twin ewes respectively. Milder energy deficits do not usually result in a reduction in lamb birth weight and are met by the maternal body, reflecting the ability of the ewe to maintain foetal growth at the expense of her own body tissues (Robinson, 1977). The extent to which ewes can utilize body tissue to maintain foetal growth varies both between experiments and breed (Robinson and McDonald, 1979).

In a review of the literature, Robinson (1982 and 1983a) drew attention to the wide variation in the amounts of metabolisable energy (ME) that caused a standard reduction in lamb birth weight (Table 1.3). For example, the range in ME intakes found to cause a 20% reduction in lamb birth weight varied from 250 to 450 KJ/kg$W^{0.75}$/day (Robinson, 1982 and 1983a). Some of this variation is undoubtedly due to differences in maternal reserves, ewe and lamb genotype and diet composition.

Table 1.3 *Ranges in ME intakes (kJ/kg $W^{0.75}$/day) in late pregnancy associated with a given reduction in lamb birth weight*

% reduction in lamb birth weight	Range in ME intakes (kJ/kg$W^{0.75}$/day)
0	530 to 950
10	340 to 560
20	250 to 450
25	210 to 405

(From data sources reviewed by Robinson, 1982 and 1983a).

1.3.2.2 Efficiency of utilisation of ME for growth of the concepta

Estimates of the efficiency of utilisation of ME for foetal growth are low (Graham, 1964; Russel *et al.*, 1967b; Sykes and Field, 1972) and range from between 0.05 to 0.22. This

compares with values of ME utilisation for growing lambs of between 0.43 and 0.53 (Wilkinson and Greenhalgh, 1995). A low efficiency of ME conversion into foetal growth, along with the rapid weight gains of the foetus in late pregnancy results in the high nutrient requirements of late pregnant ewes observed in practice. The inefficiency by which ME is used for pregnancy is due to the high heat production and oxygen consumption of the foetus and placenta (Rattray, 1992).

Estimation of the energy requirement of pregnant ewes is complicated by the low level of energetic efficiency of conceptus growth and the large variation in estimates of this efficiency (Rattray, 1974; Robinson *et al.*, 1980). Robinson *et al.* (1980) combined their own data on the subject with comparable experiments from the literature to find that there appeared to be a positive relationship between the efficiency of utilisation of ME for growth of the concepta (K_c) and the ME concentration of the diet. The value of K_c was calculated to be 0.145 for diets with a ME concentration of 10.5 MJ/kgDM and the slope of the regression of K_c on ME concentration (MJ/kgDM) was 0.029. These observations imply that some of the variation in the relationship between ME intake and lamb birth weight was due to the ME concentration of the diet. Division of the daily rates of energy deposition in the gravid uterus by the coefficient 0.145 for K_c provides an estimates of the energy requirements of conceptus growth for ewes fed a diet containing 10.5 MJ of ME/kgDM (Robinson, 1983a; Table 1.4). In diets with energy concentrations other than 10.5 MJ/kgDM, the value of K_c can be adjusted using the value of 0.029/MJ of ME (Robinson, 1983a). Robinson (1983b) reported that when dietary ME concentrations fall below 10 MJ/kgDM, energy derived from maternal tissues is used more efficiently than that of dietary origin.

Table 1.4 *Estimates of the daily metabolisable energy requirements (MJ of ME/kg lamb birth weight) above maintenance for conceptus growth (ME_p) in relation to stage of gestation in ewes receiving diets containing two differing energy concentrations*

ME concentration of the diet	Stage of gestation (days)				
(MJ/kgDM)	88	102	116	130	144
10.5	0.23	0.37	0.54	0.72	0.88
9.0	0.33	0.53	0.77	1.02	1.25

(Robinson, 1983b).

Differences in K_c due to litter size are small in comparison with those which appear to be linked with the ME concentration of the diet (Robinson *et al.*, 1983a). Estimates of the daily ME requirements (MJ of ME/kg lamb birth weight) above maintenance for conceptus growth (ME_p) for ewes at 130 days of pregnancy were 0.68 to 0.77 for ewes with a litter size of four and one respectively (Robinson *et al.*, 1983a; Table 1.5).

Table 1.5 *Estimates of the daily metabolisable energy requirements (MJ of ME/kg lamb birth weight) above maintenance for conceptus growth (ME_p) in relation to litter size and stage of gestation in ewes receiving diets containing 10.5 MJ of ME/kg dry matter*

Number of foetuses	Stage of gestation (days)						
	60	74	88	102	116	130	144
1	0.10	0.15	0.23	0.38	0.57	0.77	0.97
2	0.10	0.15	0.23	0.37	0.55	0.73	0.90
3	0.10	0.15	0.23	0.37	0.54	0.70	0.85
4	0.10	0.15	0.23	0.36	0.52	0.68	0.81

(Robinson, 1983a).

ARC (1980) give the daily energy retention in the gravid uterus as:

E_c, MJ/d = $0.25W_0(E_t \times 0.07372e^{-0.00643t})$

Where E_c is the daily energy retention (MJ/d), W_0 is the total weight of lambs at birth (kg), t is the number of days from conception and $Log_{10}E_t$ (MJ/d) = $3.322-4.979e^{-0.00643t}$

ARC (1980) assigned a constant value of 0.133 to the efficiency of use of ME for growth of the concepta (K_c) and the effect of the ME concentration of the diet reported by Robinson (1983b) was not accounted for. The calculated ARC (1980) estimates of the daily ME requirements (MJ of ME/kg lamb birth weight) above maintenance for conceptus growth (ME_p) are presented in Table 1.6. In addition to the ME requirements for pregnancy, the requirements for maternal maintenance and wool growth are also given in Table 1.6 and together they will quantify the overall energy needs for a ewe in late pregnancy (ARC, 1980).

Table 1.6 *Estimates of the daily metabolisable energy requirements (MJ/d) for maintenance, conceptus growth and wool growth at different stages of gestation for ewes fed a diet of 0.59 metabolisability (q_m), weighing 70 kg liveweight and having lambs with a total birthweight of 8.2 kg*

ME requirement	Stage of gestation (days)				
(MJ/d)	88	102	116	130	144
Maintenance	7.94	7.94	7.94	7.94	7.94
Conceptus growth	2.01	3.23	4.92	7.18	10.07
Wool growth	0.13	0.13	0.13	0.13	0.13
Total	10.08	11.30	12.99	15.25	18.14

(adapted from ARC, 1980)

1.3.2 Protein requirements

In addition to protein deposition in the gravid uterus, mammary development and colostrum synthesis takes place in the final weeks of gestation in preparation for lambing. As a consequence, the net protein requirement of the ewe will increase rapidly over the same period. Not surprisingly, undernutrition during this period will therefore reduce mammary development and colostrum production (Mellor, 1987).

AFRC (1993) presents the daily metabolisable protein (MP) requirement for conceptus growth (MP_c; g/d) in sheep to produce a lamb of weight (W_0; kg) as:

$$MP_c \text{ (g/d)} = 0.25W_0 (0.079TP_t \times e^{-0.00601t})$$

where t is the number of days from conception and TP_t in g is given by ARC (1980) as:

$$\log_{10} (TP_t) = 4.928 - 4.873e^{-0.00601t}$$

The MP requirements for pregnancy, together with the requirements for maintenance quantifies the overall protein requirements for a ewe in late pregnancy (ARC, 1980; Table 1.7).

Table 1.7 *Estimates of the daily metabolisable protein requirements (g/d) above maintenance for conceptus growth and maintenance at different stages of gestation for ewes weighing 70 kg liveweight and having lambs with a total birthweight of 8.2 kg*

MP requirement	Stage of gestation (days)				
(g/d)	88	102	116	130	144
Maintenance	73	73	73	73	73
Conceptus growth	11	17	26	37	51
Total	84	90	99	110	124

The equations of ARC (1980), that are used to calculate the MP requirement for pregnancy

were adopted by AFRC (1993) but do not take into consideration the protein requirements for udder development or for colostrum production and therefore the values shown in Table 1.7 are likely to be an under estimation of actual requirements in the last weeks of pregnancy. For the same reason, Robinson (1987) reported that the requirement for undegradable protein in twin-bearing ewes would be higher and would be required earlier than previous estimates by ARC (1980).

1.3.3 Interaction between energy and protein supply

It is widely accepted that ruminant diets must contain a minimum amount of ERDP per megajoule (MJ) of FME to provide maximal synthesis of microbial protein (AFRC, 1993). Ewes in late pregnancy and early lactation are predicted to require a minimum of 11 g ERDP/MJ of FME and this amount is reduced at lower outflow rates where fermentable energy is used less efficiently due to a re-cycling of microbial protein within the rumen (Newbold, 1994). A reduction in either ERDP or FME supply below the stated optimum would lead to reductions in microbial protein and thus total MP supply to the ewe (AFRC, 1993). Robinson (1983a) calculated that diets which meet the ewes energy requirement and which contain adequate protein for maximal synthesis of microbial protein, will provide the ewe with enough microbial plus undegraded dietary protein to meet the net protein requirements of the ewe up to about three weeks before lambing. A supplement of dietary protein of low rumen degradability is required if fermentable energy intake is limiting microbial protein production or if the ewes requirement for net protein exceeds that which can be supplied from the basal diet, a situation commonly seen in late pregnancy or early lactation (Robinson, 1983a). Table 1.8 shows the rapid increase in the net accretions of protein in the gravid uterus and udder in relation to the corresponding ME requirements, rising from 5.3 to 11.7 g/MJ for twin bearing ewes at 102 and 144 days of pregnancy respectively.

Table 1.8 *The net accretions of crude protein in the gravid uterus and udder in relation to the corresponding ME requirements (g/MJ) for ewes expecting varying litter sizes and at different stages of gestation*

Number of foetuses	Stage of gestation (days)						
	60	74	88	102	116	130	144
1				5.2	5.2	6.0	11.3
2	5.2	5.2	5.2	5.3	5.3	6.1	11.7
3				5.3	5.3	6.2	12.1
4				5.3	5.4	6.2	12.6

(Robinson, 1983a).

In most commercial systems of sheep production it is extremely difficult to meet the entire requirements for energy in late pregnancy from dietary sources alone (Robinson, 1983a). A reduction in energy intake (and hence FME) will result in a lower contribution of microbial protein to the net protein needs of the ewe. Supplementation of feeds with protein sources of low rumen degradability have found to be beneficial in alleviating the effects of low ME diets in late pregnant ewes (Robinson, 1977) and provides an alternative to high levels of concentrate feeding which can be detrimental to rumen function (Robinson, 1987). The amount of supplementary undegradable dietary protein (UDP) required increases with advancing pregnancy and with decreasing ME supplied by the diet (Robinson, 1990a; Figure 1.3).

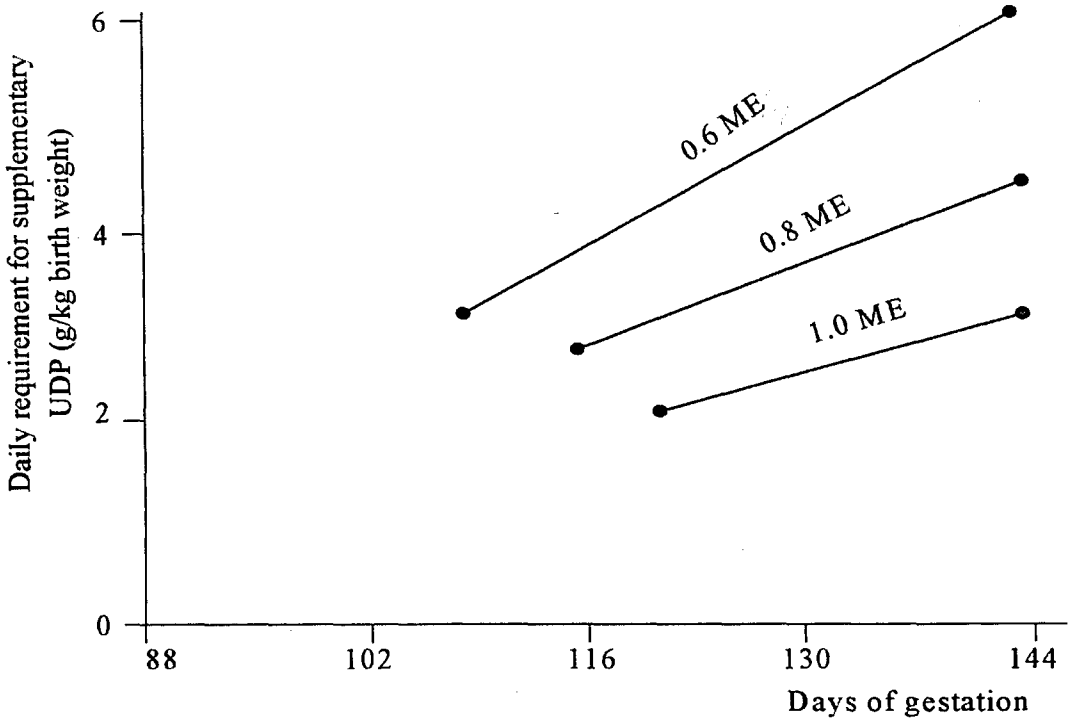

Figure 1.3 The daily amounts of supplementary undegradable dietary protein (UDP) required by ewes receiving in their diet, all their energy needs for pregnancy (1.0 ME), 80% of their needs (0.8 ME) or 60% of their needs (0.6 ME). The amounts of UDP refer to requirements above the microbial and UDP supplied by a basal diet containing 10 g crude protein per MJ ME. (From Robinson, 1990a).

It follows from Figure 1.3 that the stimulatory effect of UDP on lamb birth weight is proportional to the degree of the energy deficit (Robinson and McDonald, 1989). In addition, Robinson and McDonald (1989) stated that ewes consuming adequate ME to meet the maintenance needs of the maternal body in late pregnancy would increase the lamb birth weight of twin lambs by 25% from supplementing the basal diet containing 10 g crude protein (CP)/MJ of ME with a similar quantity of protein from fishmeal or heat-treated soya-bean meal. There are also increases in the volume of colostrum produced and by implication improvements in lamb survival when this inter-relationship between energy and protein intake in late pregnancy is exploited (Robinson, 1987).

1.4 COLOSTRUM PRODUCTION

1.4.1 Introduction

Colostrum is the initial secretion in the lactation of the ewe and is normally available to the newborn immediately after parturition (Pattinson *et al.*, 1995). It has higher concentrations of fat, protein and lower concentrations of lactose than normal milk (Perrin, 1958). Colostrum contains high concentrations of immunoglobulins (Ig; Al-Sabbagh *et al.*,1995) and is also a concentrated source of vitamins A and E, essential fatty acids, proteins and certain trace and major minerals (Rattray, 1992). There is some disagreement about how long colostrum is produced for. Estimates range from as much as 6 days *post partum* (Perrin, 1958) to 18 hours *post partum* (Mellor and Murray, 1986). In fact the transition from colostrum to milk appears to be gradual and is accompanied by a marked decrease in the concentrations of total solids, protein, ash, immunoglobulin G (IgG; Pattinson *et al.*, 1995; Table 1.9) and an increase in lactose concentration (Mellor, 1990).

Table 1.9 *Colostrum composition (g/l) in Suffolk X Cambridge ewes over the first 24 hours post partum*

hours *post partum*	Total solids	Fat	Protein	Ash	IgG
1	367	132	203	9.6	116
9-12	285	135	109	7.2	47
21-24	242	122	71	6.8	15

(adapted from Pattinson *et al.*, 1995)

1.4.2 Colostrum requirements

At the point of birth, energy for the maintenance of the lambs body temperature comes from the metabolism of body tissues (Mellor and Cockburn, 1986) and subsequently energy yielding substrates in colostrum and later milk take over. Consumption of colostrum results in an

increased heat production capacity of lambs following feeding (Eales and Small, 1981). Therefore, the amount of colostrum that a lamb requires depends largely on the amount needed for heat production (Mellor and Murray, 1986). Factors which increase the lambs rate of heat production (eg. cold exposure) will increase the requirement for colostrum. Any lamb exposed to temperatures between 0 - 10°C would become hypothermic during the first 24 hours of life unless they received about 280 g colostrum / kg birth weight (Mellor and Murray, 1985b).

Pattinson *et al.* (1995) calculated that lambs born indoors at temperatures between 2°C and 10°C would require between 143 and 175 g of average quality colostrum per kilogram of bodyweight for maintenance during the first 24 hours of life. Due to the large variation in the composition of colostrum produced in the experiment of Pattinson *et al.* (1995), it is interesting to note that for lambs born at the same temperatures to ewes producing energy concentrations in colostrum which were 1 standard deviation below the mean would require between 187 and 229 g colostrum / kg bodyweight (Pattinson *et al.*, 1995).

Shubber *et al.* (1979a) reported that single lambs consumed 35% more colostrum than twins and 60% more than triplets during the first 18 hours *post partum* and that those lambs which consumed the most colostrum also had the highest liveweight gain. This immediate effect is likely to be carried over into the first few weeks of the lambs life (Khalaf *et al.*, 1979b). Actual consumption for singles, twins and triplets were 195, 165 and 125 g/kg respectively (Shubber *et al.*, 1979a) and only the triplets showed a significant mortality rate (17%). This observation supports estimates of colostrum requirements by other authors (Mellor and Murray, 1986; Pattinson *et al.*, 1995).

1.4.2.1 Immunoglobulin concentration in lambs

Lambs are born hypoimmunocompetent (Bramwell, 1970) and are therefore dependent on colostrum to supply maternal immunoglobulins (Ig). In a study by Hunter *et al.* (1977) only 7 out of 48 lambs had measurable IgG levels at birth, but this had risen to 25.3 mg/ml by 8 hours *post partum* and 35.6 mg/ml by 24 hours, an increase due to the transfer of intact IgG from ingested colostrum. In sheep, IgG constitutes 92% of the total immunoglobulins (Ig) in colostrum, whereas IgA and IgM represent about 6% and 2 % respectively (Smith *et al.*, 1975). Approximately 87% of the total IgG in ewes colostrum is IgG_1, the remaining 13% being IgG_2. The actual concentration of IgG in ewes colostrum at birth varies greatly. Al-Sabbagh *et al.* (1995) reported a mean concentration of 79 mg/ml, but individual samples ranged from a low of 14 mg/ml to a maximum of 114 mg/ml. Immunoglobulin concentration in ewes colostrum falls rapidly after parturition (Hunter *et al.*, 1977; Shubber *et al.*, 1979b; Al-Sabbagh *et-al.*, 1995; Pattinson *et al.*, 1995; Table 1.9). Shubber *et al.* (1979b) collected colostrum samples at 6 hour intervals up to 48 hours *post partum* and concluded that total Ig (IgG_1, IgG_2, IgM and IgA) concentrations were very low by 36 hours after parturition. Al-Sabbagh *et al.* (1995) analysed samples of colostrum taken up to 12 hours *post partum* and predicted IgG concentrations would decrease to zero by 23 hours *post partum*. This reduction in IgG concentration, combined with the reducing ability of the lamb to absorb immunoglobulins across the intestinal wall with increasing age (Klobosa *et al.*, 1985) gives importance to the early intake of colostrum.

The total amount of Ig produced largely depends on the volume of colostrum produced (Shubber *et al.*, 1979b; O'Doherty and Crosby, 1997). Shubber *et al.* (1979b) and O'Doherty and Crosby (1997) found a positive relationship between total colostrum yield and the total Ig yield. However the same authors found that Ig concentration in colostrum was reduced with

increasing yield. There is a clear correlation between the amount of Ig ingested by lambs and their subsequent plasma Ig levels (Shubber *et al.*, 1979b; Parker and Nicol, 1990; O'Doherty and Crosby, 1997). Lamb plasma Ig concentration reaches a maximum at levels of intake of 210 ml colostrum/kg birthweight and plateaus at around 24 hours after the first feed (Parker and Nicol, 1990), an observation probably due to the closure of the lambs intestinal wall to Ig at around this time (Klobosa *et al.*, 1985). The closure of the lambs gut to intact molecules appears to occur between 24 and 48 hours *post partum* (Campbell *et al.*, 1977) and may be related to the replacement of epithelial cells of the small intestine with parietal cells over the first three days after birth (Hill, 1956). Shubber *et al.*, (1979b) found that about 0.20 to 0.25 of Ig ingested was present in the lambs plasma at around 30 hours after the first feed of colostrum. These figures broadly agreed with Parker and Nicol (1990) and O'Doherty and Crosby (1997) who reported that 0.27 and 0.17 respectively of the Ig ingested was present in the lambs plasma at 24 hours from the first feed. The levels present in the plasma did not, however correlate directly with ingestion rate (Shubber *et al.*, 1979). Single lambs ingested nearly 100% more than triplets, but the levels in the plasma at 30 hours post feeding was only around 7% higher. There appears to be a decrease in the efficiency of absorption of Ig as the amount ingested increases. Hunter *et al.* (1977) found no difference in lamb plasma IgG between the first and second born of twins at 24 hours *post partum*, except in lambs born to ewes with low yields of colostrum, where colostrum available to the second born was limiting its Ig intake.

Parker and Nicol (1990) found a high degree of variation in lamb plasma IgG in lambs receiving the same amount of colostrum. Some of the variation within breeds is due to gestation length, with longer pregnancies producing higher concentrations of colostral Ig (Halliday, 1974). Variation in lamb plasma IgG concentrations can also originate from differences in total yield

of colostrum produced by the dam. Hunter *et al.* (1977) found that the highest intakes and hence highest plasma concentrations of Ig were found in lambs from ewes with medium yields of colostrum. In high yielding ewes, low colostral Ig concentration limited Ig intake and hence plasma concentrations.

1.4.3 Nutritional effects on colostrum production

A major factor influencing the availability of colostrum is the level of ewe nutrition during late pregnancy (Mellor and Murray, 1986). In sheep at term, the weight of the mammary tissue averages 0.3 - 0.4 of the total lamb weight for litter sizes of 2 to 4 (Mellor and Murray, 1985a), which indicates that the metabolic cost of mammary development is less than that of the foetal growth. However, proportionally 0.7 of this increase in weight occurs in the last 4 weeks of pregnancy (Robinson *et al.*, 1978). It can be calculated that the energy cost over this latter part of pregnancy is similar for mammary growth and for foetal development and it is therefore not surprising that maternal undernutrition during this time would result in a reduction in mammary growth (Mellor and Murray, 1985a).

The growth of virtually all the lobule alveolar epithelial cell system of the mammary gland in ewes takes place during the last trimester of pregnancy (Forsyth, 1986). Mammary growth rate increases progressively during late pregnancy, showing marked increases in the last five days before parturition (Mellor and Murray, 1985a). In contrast to this, foetal growth rate decreases in the same period and approaches zero during the last five days of pregnancy (Mellor and Murray, 1981). It is well known that the deleterious effects of maternal under nutrition in late pregnancy can be partially overcome by adequate intakes of colostrum at birth (Mellor and Murray, 1985b). This is important in nutritionally deprived ewes because, although, previously under fed ewes do not have the ability to promote foetal growth when re-fed in the last 14 days

of pregnancy, they can promote mammary growth just before parturition (Mellor and Murray, 1985b).

High levels of progesterone are responsible for the growth and development of the mammary gland during pregnancy (Cowie and Tindal, 1971). Development of the alveoli and ducts requires several hormones including oestrogens and progesterone and, during the latter part of pregnancy prolactin and glucocorticoids are required for the full maturation of the mammary glands. At parturition the mammary gland switches from colostrum production to milk secretion, the crucial hormonal changes being a decrease in the level of progesterone and an increase in the level of prolactin and glucocorticoid levels (Mellor *et al.*, 1987). The data of Mellor *et al.* (1987) demonstrated that underfed ewes had delayed onset of milk secretion compared to well fed ewes, which corresponded to a delay in the fall of plasma progesterone levels. However, the experimental design failed to separate the effects of nutritional state and those of falling plasma progesterone levels and so it is impossible to determine if the observed effects were due to the lack of nutrients for synthesis of udder secretions or an effect of delayed progesterone withdrawal. In the same experiment, Mellor *et al.* (1987) reported that re-feeding previously underfed ewes from five days *pre partum* returned the secretory functions of the udder to those observed in well-fed ewes. The delayed onset of lactogenesis was also observed by Mellor and Murray (1986) who found that whilst colostrum yield appeared to be similar at each milking for well fed ewes during the first 18 hours *post partum*, underfed ewes initially had small yields, which subsequently increased at each milking.

In well nourished ewes, colostrum accumulates in the udder during the last few days of gestation, thereby ensuring an adequate supply of colostrum for the new-born lambs (Robinson, 1990b). Undernutrition during this time reduces the weight of secretory tissue at parturition

(Oddy *et al.*, 1984) and not only reduces the initial colostrum yield but the subsequent secretion rates and the available constituents in colostrum (Mellor and Murray, 1985a, 1985b). Hall and Egan (1988) reported that ewes with a higher energy intake over the final 30 days *pre partum,* had higher colostrum yields. A ewe on a low plane of nutrition in late pregnancy (plasma glucose = 1.0 to 1.5 mmol/l) would have about half of the colostral constituents available to her lambs as a ewe on a high plane of nutrition (plasma glucose = 2.6 to 3.3 mmol/l; Mellor and Murray, 1985b). Mellor and Murray (1986) measured total colostrum yields that accumulated up to 18 hours *post partum* of 2078 and 994 ml for well fed (condition score 3 to 4 at term) and underfed (condition score 1.5 to 2 at term) twin-bearing ewes respectively.

Undernutrition in late pregnancy not only reduces the available colostrum to the newborn lamb, but also significantly reduces the lambs body lipid concentration at birth (Mellor and Murray, 1985b) and increases the surface area:body weight ratio, thus increasing the chance of hypothermia (Robinson, 1990b). Hypothermia in newborn lambs is prevented by the metabolism of predominantly body lipid (Mellor and Cockburn, 1986) and by the consumption of colostrum. However, Mellor and Murray (1985b) reported that increasing the plane of nutrition after day 131 of pregnancy to previously undernourished ewes had no effect on lamb body lipid concentration due to the low rates of placental fatty acid transfer (Elphick *et al.*, 1979). The amount of colostrum which must be consumed to meet energy requirements of newborn lambs is higher than the amount needed to meet the Ig requirements (Pattinson *et al.*, 1995), resulting in lambs born to undernourished ewes being more likely to die from hypothermia. However, this may not be the case when lambs are kept at warm temperatures or when the colostrum fed was produced after 12 hours *post partum* (Pattinson *et al.*, 1995). Poor maternal nutrition alone is unlikely to affect the lamb plasma IgG concentration and hence the ability of the lamb to fight infections (Khalaf *et al.* (1979a). It is only when poor ewe

nutrition is combined with deprivation of colostrum in early life that a significant drop in lamb plasma Ig concentration is seen (Khalaf *et al.* (1979b).

1.4.3.1 The effects of digestible undegradable protein on colostrum production

In late pregnancy, due to the rapid daily liveweight gains of the foetus, the synthesis of colostrum and the reduction in dry matter intake, the ewe may become carbohydrate deficient (Russel *et al.*, 1967a). The detrimental effects of a maternal energy deficit in late pregnancy on the colostrum production at lambing may be ameliorated through dietary supplements of digestible undegradable protein (DUP; McPherson *et al.*, unpublished data quoted in Robinson, 1987). For example, increasing the mean daily intake of CP from 80 to 128 g/d for twin-bearing ewes with a daily ME intake of 8.1 MJ of ME / day, increased the colostrum production during the first 24 hours *post partum* from 1.02 to 1.58 kg (Table 1.10). Increases in DUP supply may cause improvements in the yield of colostrum by increasing the amino acid supply to the mammary gland and causing ewes to respond by increasing the mobilisation of adipose tissue, or alternatively, the additional amino acids may be used as energy precursors for the TCA cycle.

Table 1.10 *The effect of increasing crude protein (CP) intake in late pregnancy by the addition of white fish meal to diets supplying two intakes of energy on the colostrum production (kg) of twin-bearing Finn Dorset ewes*

Mean daily intake of ME (MJ)	8.1	8.1	14.5	14.5
Mean daily intake of CP (g)	80	128	128	185
Colostrum production (kg):-				
First 3 hours after lambing	0.15	0.32	0.38	0.64
First 24 h after lambing	1.02	1.58	1.89	2.10

(Robinson, 1987).

In contrast to the results of Robinson (1987), Pattinson *et al.* (1991) found that protein concentration (111 or 144 gCP/kg DM) in the diet of ewes had no significant effect on colostrum yield or composition at birth or at 12 to 16 hours *post partum*. In agreement with Pattinson *et al.* (1991), Dawson *et al.* (1999) found that increasing the UDP supply from 24.0 g/d to 49.8 g/d during the 6 weeks prior to lambing had no effect on colostrum yield or quality. O'Doherty and Crosby (1997) found that addition of soya-bean meal protein to a basal diet of formic-acid treated grass silage fed to ewes in late pregnancy, resulted in significant improvements in initial colostrum yield. However, in the study of O'Doherty and Crosby (1997) additions of soya-bean meal inevitably lead to increases in the ME intake of the ewes as well and therefore any response can not be attributed to increases in protein supply. The recent studies of Pattinson *et al.* (1991) and O'Doherty and Crosby (1997) support the observations made by Robinson (1987) in that the response to DUP appears to be greatest when the ME intake of the ewe is low. Ewes in the experiments of Pattinson *et al.* (1991) where no effect of supplementing with dietary crude protein or DUP was seen, had a high ME intake (24.6 MJ ME/day), whilst in the study of O'Doherty and Crosby (1997) ME intakes were much lower (10.4 MJ ME/day).

1.4.3.2 Additional factors affecting colostrum production

Dam breed has been shown to affect colostrum yield in cattle (Kruse, 1970) and in ewes (Pattinson and Thomas, 1998). Pattinson and Thomas (1998) reported that milk type ewes (Friesland X Lleyn) had a higher yield of colostrum and a lower IgG concentration at 12-16 hours *post partum* than meat type ewes (Charollais X Lleyn and Charollais X Cambridge). Dam breed has also been shown to affect early lamb mortality (Sidwell and Miller, 1971). It is possible that this effect, at least in part, is due to the quantity and quality of colostrum produced (Mellor and Murray, 1986).

The effects of litter size on colostrum production are inconclusive. Shubber *et al.* (1979a) reported that colostrum production was higher in twin bearing than either single or triplet bearing ewes. Hall and Egan (1988) also reported that twin bearing ewes had a significantly higher colostrum yield at 1 hour *post partum* than single bearing ewes, but there was no further increase in yield with triplet bearing ewes. Pattinson *et al.* (1995) concluded that litter size of Cambridge and Suffolk x Cambridge ewes had no effect on colostrum yield at birth, but those producing large litters had higher subsequent secretion rates at 3-6 and 12-16 hours *post partum*. In contrast to the above reports, Alexander and Davies (1959) reported that a higher proportion of twin bearing, compared to single bearing ewes, had no colostrum at birth and had a lower secretion rate at 12-14 hours *post partum* (McCance and Alexander, 1959). In addition, Hall *et al.* (1989), in an experiment with adult Border Leicester X Merino ewes fed grass hay (8.1 MJ of ME/kgDM) to appetite from day 109 of pregnancy reported that singles, twins and triplets gave 218, 141 and 0 g of colostrum at 1 hour *post partum* respectively. Other authors have reported no effect of litter size on initial colostrum production (Thomas *et al.*, 1988) or on subsequent secretion rates (McNeill *et al.*, 1988).

The method by which colostrum was measured and the adequacy of late pregnancy nutrition differed between the experiments reported and could account for the conflicting observations of the effects of litter size on both colostrum yield and composition made by researchers. For example, Shubber *et al.* (1979a; 1979b) measured the colostrum produced by allowing the lamb to suckle and using pre and post sucking weights to calculate consumption. This method depends as much on the sucking vigour and appetite of the lamb as it does on the ability of the ewe to produce colostrum (Shubber *et al.*, 1979b). Other workers reported here expressed milk manually, a method which removes lamb differences. Different responses to increasing litter size were reported by Hall *et al.* (1989) and Hall and Egan (1988). Pregnant ewes in the

experiment of Hall *et al.* (1989) had low nutrient intakes. The *ad libitum* hay diets given caused ewes to have a high liveweight losses and have elevated plasma betahydroxybutyrate (BHB) concentrations and also showed dramatic reductions in the initial colostrum yield with increasing litter size. These observations are not surprising given that the diets only supplied about half of the daily ME requirements quoted by AFRC (1993). Conversely, Hall and Egan (1988) fed restricted hay or lucerne diets, supplemented with oat grain, barley or infusions of glucose. The supplementation would imply (although not detailed) an improved ME intake above that of Hall *et al.* (1989). In contrast to Hall *et al.* (1989), Hall and Egan (1988) showed improved initial yields of colostrum in twin bearing ewes compared to singles. Mellor (1990) concluded that in ewes that are well fed, more colostrum is produced as the number of lambs carried increases in order to provide enough colostrum for the extra lamb(s). However, in underfed ewes the heavy nutrient demand associated with larger litter sizes often results in production of less colostrum when feed quality and or quantity is low.

1.5 NUTRITION DURING EARLY LACTATION

1.5.1 Milk yield and lamb growth

Feeding strategies for late pregnancy that recognise the needs for diets to supply increasing amounts of rumen undegradable protein (UDP) as the difference between ME requirement and ME intake increases are now accepted in practice (Robinson, 1987). Similarly, in lactation ewes can seldom consume enough food to meet their nutritional requirements for milk production and subsequently mobilize body reserves to produce a milk yield nearer to their genetic potential (Cowan *et al.*, 1980).

Milk production in the ewe peaks at around 2 to 3 kg/day in the second and third weeks of lactation and subsequently declines thereafter to about 1 kg/day at around 12 weeks of lactation (Rattray, 1992). In the first weeks of lactation when the lamb is completely dependent on milk, growth rate is almost entirely determined by differences in milk intake (Doney *et al.*, 1981). Data presented by Robinson (1983b; Figure 1.4) shows the empirical relationship between milk yield and lamb growth rate and illustrates that increases in lamb growth rates are seen with improvements in milk yield and with decreases in the number of lambs being suckled. Peart (1982) also demonstrated the reduced efficiency of converting milk into lamb growth at high milk intakes. Proportional increases in milk yield of 0.20 to 0.50 and 0.35 to 0.70 above that of ewes suckling single lambs have been reported for ewes suckling twins and triplets respectively (Treacher, 1983; Rattray, 1992) and the differences appear to be mediated through an increase in udder size and not secretory efficiency (Davis *et al.*, 1980). The yield of milk could be related to the stimulatory effects of suckling on prolactin and oxytocin (Loerch *et al.*, 1985; Treacher, 1983). Gootwine and Pollott (2000) reported that litter size significantly influenced the total milk yield for the lactation in Awassi dairy ewes, even though the lambs had been removed from the ewes within 24 hours of birth. Butler *et al.* (1981) suggested that udder

development and consequently milk production in sheep is affected by the number of foetuses carried by the ewe and Byatt *et al.* (1992) reported that this may be mediated through the relatively high concentration of maternal placental lactogen found in ewes bearing multiple foetuses.

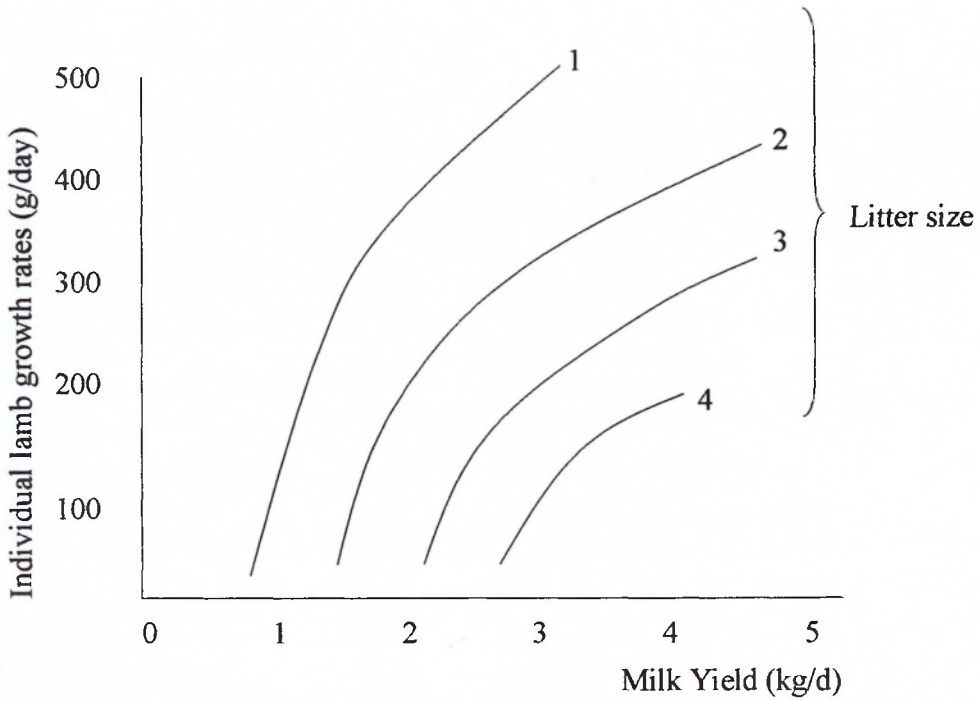

Figure 1.4 Relationship between milk yield and lamb growth rate (From Robinson, 1983b).

Given that mammary secretory tissue develops during late gestation (Treacher, 1983) with virtually all of the lobule-alveolar epithelial cell system being formed prior to parturition (Robinson, 1990b) and that milk production is highly correlated with the weight of secretory tissue (Oddey *et al.*, 1984), it is not surprising that late pregnancy nutrition has an effect on lactational milk yield. Wallace (1948) obtained greater yields of milk from ewes which had been well fed compared with those subjected to a low plane of nutrition in late pregnancy. In work done with lactating dairy goats, Sahlu *et al.* (1995) demonstrated that milk production in the subsequent lactation increased linearly from 2.63 to 3.05 and 3.26 kg/d respectively when *ad libitum* diets containing 7.5, 9.1 and 10.6 MJ/kg of DM were offered during late pregnancy.

Severe undernutrition in late pregnancy, as discussed earlier, can also delay the onset of lactation (Thomson and Thomson, 1953 ; Mellor *et al.*, 1987) and this effect can be mediated through a delay in the *pre partum* fall of progesterone as well as a deficient supply of dietary nutrients (Rattray, 1992).

1.5.2 Energy requirements

The ME requirement for lactation (M_l) is given by AFRC (1993) as :

M_l (MJ/d) = (Y x [EV_l])/K_l

where Y is the yield of milk in kg/d; EV_l is the energy content of ewes milk (MJ/kg) and K_l is the efficiency of utilisation of ME for milk production.

The energy requirements for milk production, maternal body maintenance and for wool growth, less the energy derived from the mobilisation of body fat quantifies the overall energy requirement for the ewe during lactation (Table 1.11; AFRC, 1993).

Table 1.11 *The ME (MJ/day) requirements of a 80 kg housed, lactating ewe fed a diet of M/D of 11.5 MJ/KgDM,* q_m *=0.61*

	Milk Yield (kg/day)					
	1.0		2.0		3.0	
Liveweight loss (g/day)	DMI	ME	DMI	ME	DMI	ME
0	1.5	17.5	2.2	25.6	2.9	33.9
-50	1.4	15.8	2.1	23.8	2.8	32.0
-100	1.2	14.0	1.9	22.0	2.6	30.2

(adapted from AFRC, 1993; DMI = dry matter intake).

1.5.2.1 Maternal body fat mobilisation

At lambing it is desirable to have lowland ewes at body condition score (BCS) 2.5-3 (MLC, 1981) to allow losses in the first six weeks of lactation of up to 1 BCS (Robinson *et al.*, 1983b). This practice is common on UK farms, utilizing maternal fat as an energy source when dietary nutrients are typically expensive and allowing the ewe to re-build condition on inexpensive pastures in the late summer and autumn (Russel, 1984). The total ME requirement of an 80 kg ewe which is yielding 3 kg of milk per day is approximately double that of the same ewe which is yielding 1 kg of milk per day (Table 1.11; AFRC, 1993). Table 1.11 also demonstrates that the requirement for ME is reduced if the ewe is in sufficient body condition to allow some mobilisation of adipose tissue. AFRC (1993) estimates the milk yield of a lowland ewe during the first month of lactation to be 3 kg/d and such ewes will invariably draw on body fat reserves even when offered high quality food *ad libitum* (Cowan *et al.*, 1980).

The factors that control the rate of fat loss and hence its contribution to milk production are the amount of maternal fat present and the level of ME intake (Robinson, 1987; Robinson, 1990a). The influence of these two factors are presented in Table 1.12, which shows the contribution that maternal fat can make to milk yield. However, Table 1.12 also demonstrates that the use of maternal fat as an alternative to dietary energy means that milk production will fall short of the ewes potential production. In addition to responses of lactating ewes to energy supply, data from Gonzalez *et al.* (1982) and Ngongoni *et al.* (1989) have shown that for any given level of ME intake and body fatness the milk yield produced depends on the quantity and quality (amino acid profile available for absorption at the small intestine) of dietary protein given.

Table 1.12 *The effect on rate of body fat loss, milk production and lamb growth rate in 70 kg, twin-suckling ewes with a ME intake of 20, 25 or 30 MJ/day and a body fatness of 5, 10, 15 or 20 kg of body fat*

ME intake (MJ/day)	Body fat (kg)	Body fat loss (g/day)	Milk Yield (kg/day)	Growth rate (g/day)
20	5	105	2.1	180
	10	190	2.4	220
	15	275	2.6	260
	20	360	2.8	280
25	5	60	2.6	260
	10	105	2.8	280
	15	150	2.9	290
	20	190	3.1	310
30	5	20	3.3	340
	10	20	3.3	340
	15	20	3.3	340
	20	20	3.3	340

(From Robinson, 1990a).

1.5.3 Protein requirements

AFRC (1993) predicted the MP requirement for milk production (MP_l) as:

MP_l (g/d) = Y (TP_m/k_{nl})

where Y is the milk yield (kg/d), TP_m is the true protein content of sheep milk (48.9 g true protein / kg milk) and k_{nl} is the efficiency of utilisation of absorbed amino acids for milk protein synthesis (0.68).

The MP requirements for milk production, maternal body maintenance and for wool growth, less the MP derived from liveweight loss quantifies the overall MP requirement for the ewe during lactation (Table 1.13; AFRC, 1993).

Table 1.13 *The MP (g/day) requirements of a 80 kg housed, lactating ewe*

	Milk yield (kg/d)		
	1.0	2.0	3.0
Liveweight loss (g/day)			
0	158	234	309
-50	152	228	303
-100	146	221	297

(adapted from AFRC, 1993).

1.5.3.1 Production responses to digestible undegradable protein during early lactation

In an effort to quantify responses to diets with reduced protein degradability, many earlier experiments compared feeding sources of animal protein (namely fishmeal; low rumen degradability) with sources of degradable protein (Robinson *et al.*, 1979; Gonzalez *et al.*, 1982).

Robinson *et al.* (1979) compared isonitrogenous diets containing either fishmeal, soya-bean meal or groundnut meal as protein supplements for ewes in early lactation. Robinson *et al.* (1979) reported that ewes fed fishmeal in their second week of lactation produced milk yields that were 0.40 and 0.43 kg/d higher than milk yields produced by ewes fed either soya-bean meal or groundnut meal respectively. However, by week five of lactation there was no difference in the milk output of the ewes fed any of the protein supplements. The growth rate of lambs on the experiment by Robinson *et al.* (1979) reflected the relative differences in milk yield. Consequently, improved growth rates were recorded in lambs suckling ewes fed fishmeal in week two, but not in week five of lactation. The increases in milk yield were attributed to the amounts of non-ammonia nitrogen (NAN) passing the abomasum of 40.3, 35.0 and 34.1 g/d for diets containing fishmeal, soya-bean meal and ground nut meal respectively (Robinson

et al., 1979). Similarly, Gonzalez *et al.* (1982) reported that ewes in negative energy balance in early lactation responded to supplements of UDP in the form of blood meal and fishmeal by increasing their milk yield. The effect was attributed to the improved efficiency with which mobilised body fat was used for milk production when the total supply (or balance of essential amino acids) to the mammary gland were improved. In agreement with Robinson *et al.* (1979) and Gonzalez *et al.* (1982), both Purroy and Jamie (1995) and Hadjipanayiotou *et al.* (1988) reported that ewes would respond to supplements of dietary fishmeal in early lactation by increasing their milk yield. It has been demonstrated that the provision of fishmeal to lambs negative energy balance will also increase the rate of body fat mobilisation (Fattet *et al.*, 1984). Fattet *et al.* (1984) reported that lambs fed straw diets supplemented with fishmeal increased the rates of fat mobilisation and protein deposition compared to those fed straw alone, even though the total ME intake was higher in lambs fed the fishmeal supplemented diets. Fattet *et al.* (1984) suggested that an improved amino acid supply from the fishmeal supplemented diets was being used as energy pre-cursors for the TCA cycle. In addition, Sinclair *et al.* (1994) reported that twin-suckling beef cows in early lactation fed on a low ME and a high DUP diet had low plasma BHB and high plasma urea-N concentrations. Sinclair *et al.* (1994) suggested that these animals were using amino acids as energy precursors for the TCA cycle to support a higher milk yield.

A number of authors, however, have failed to see an effect of including fishmeal in the diets of lactating ewes. Jaime and Purroy (1995) found no differences in milk yield, constituent yield or subsequent growth of lambs when lactating ewes were fed concentrates containing either fishmeal, faba beans or soya-bean meal. Shaker *et al.* (1998) designed two diets for lactating ewes where the UDP supply was designed to be low (17.9% of total protein; main protein source was vetch and urea) or high (34.0% of total protein; main protein source was soya-bean

meal), but failed to observe any differences in milk yield, ewe body weight change or lamb growth rate. Several other authors working with dairy cows have reported no effect of including protein sources of differing degradability on milk yield in early lactation (Annexstad *et al.*, 1987; Robinson *et al.*, 1991; Tomlinson *et al.*, 1994).

There are a number of possible reasons for the variable response reported to supplementing diets with DUP. The quality of fishmeal is variable (Ngongoni *et al.*, 1989) and therefore any response to fishmeal would be dependent on this. In a similar experimental design to that of Robinson *et al.* (1979) and Gonzalez *et al.* (1982), Ngongoni *et al.* (1989) found no difference in the NAN reaching the abomasum or on the milk yield of ewes fed diets containing either fishmeal or soya-bean meal. The particular fishmeal used in this experiment had a much higher degradability than expected (Ngongoni *et al.*, 1989). Mehrez *et al.* (1980) investigated the processing factors that affect the degradability of fishmeal and found that the length of time within which fresh fish are stored before processing was the single biggest factor affecting its degradability in the rumen and this may explain some of the variable results observed above.

Substituting part of a dietary degradable protein for one which is largely undegradable in the rumen will lead to increases in the DUP supply (AFRC, 1993), but in some cases the reduction in rumen degradable protein supply may lead to a reduction in the efficiency of microbial protein synthesis. Thus a decrease in the contribution of microbial protein to the net protein supplied to the animal may result, and in such a situation, it is likely that no response to feeding sources of DUP would be observed.

It is also possible that responses to DUP may only be seen for ewes with an inadequate intake of ME, resulting in a negative energy balance (Robinson *et al.*, 1974). However, Gonzalez *et*

al. (1984) found that ewes (average weight of 69 kg) with a high daily ME intake (27 MJ ME/day) responded to supplements of dietary protein low in degradability by increasing milk yield, albeit with a lower incremental efficiency, than for ewes with a lower intake of ME (19 MJ ME/day). Even at the relatively high ME intakes described by Gonzalez *et al.* (1984), 27 MJ ME/day does not meet the ME requirements of a 69 kg ewe during early lactation. AFRC (1993) stated that the ME requirement of a housed, 60 kg ewe with a milk yield of 3 kg per day as 32.2 MJ/day, and thus in the experiment of Gonzalez *et al.* (1984) increases in milk production could be afforded by increases in body fat mobilisation when supplements of DUP were given. It is also logical that lactating ewes that are in poor body condition (less than body condition score 2; Russel *et al.*, 1969) at the start of lactation may not have sufficient body stores of fat to mobilise when offered supplemental DUP (Newbold, 1994).

1.6 THE USE OF BLOOD METABOLITES AS A METHOD FOR ASSESSING THE NUTRITIONAL ADEQUACY OF PREGNANT AND LACTATING EWES

The nutrient requirements of pregnant and lactating ewes are now well defined for each stage of pregnancy and lactation for ewes of different weight and birth type (AFRC, 1993). However, the intake of dry matter, metabolisable energy and metabolisable protein is variable and is often unknown under practical circumstances (Russel, 1984). This makes it difficult to determine the amount and quality of supplement required for a pre-determined level of production. It therefore seems prudent to use some measure to monitor the nutritional adequacy of a ewes diet.

The use of liveweight and body condition score change can provide some measure of the adequacy of a diet fed to pregnant and lactating ewes (Russel, 1984). Weight change is of most use in early pregnancy. After this point it is subject to the relative changes in weight of the maternal body, and of the intra-uterine contents (Russel, 1984). The weight change of a single bearing ewe will differ significantly from that of a triplet bearing ewe (Sheehan and Lawlor, 1972). Condition scoring ewes (Russel *et al.*, 1969) has the advantage that unlike weight change, it is independent of foetal numbers and foetal weight and is therefore considered more appropriate in mid pregnancy (due to large changes in placental weight) and late pregnancy (due to large changes in foetal weight) (Russel, 1984).

However, both weight change and condition score change have the distinct disadvantage of being retrospective measurements of nutrient adequacy, usually requiring a number of weeks to detect a significant difference (Russel, 1984). Once nutrient deficiency has been identified it is likely that a production penalty has occurred (Russel and Wright, 1983). Circulating blood metabolites offer a more immediate means of assessing current nutritional supply relative to

requirements (Russel, 1984).

1.6.1 Indicators of energy status

The principle blood metabolites which can be used as an indication of energy status are glucose, non-esterified fatty acids and ketones, eg beta-hydroxybutyrate, (Russel and Wright, 1983; Russel, 1984).

1.6.1.1 Glucose

Glucose is the major energy substrate for the developing foetus and for the lactating mammary gland. Glucose is supplied via the maternal blood and is synthesised mainly from propionate and amino acids in ingested nutrients (Russel *et al.*, 1967b). Everts (1990) reported plasma glucose concentrations ranging from 1.16 mmol/l in a ewe with clinical acetonaemia up to 4.73 mmol/l in a barren ewe.

During prolonged periods of energy deficit, plasma glucose concentration in ewes will decrease (Hussain *et al.*, 1996). Reductions in plasma glucose concentration have been observed in ewes on a low plane compared with a high plane of nutrition (Patterson *et al.*, 1964). In addition, glucose uptake and requirement in ewes increases as pregnancy advances (Christenson and Prior, 1978; Prior and Christenson, 1976 and 1978) especially in ewes carrying multiple foetuses (Everts, 1990). However, Russel and Wright (1983) reported that plasma glucose is a unsatisfactory index of energy status in pregnant ewes. In a comparison of blood metabolites as indicators of energy status they reported that plasma glucose is under a high degree of homeostatic control, and that changes in the rate of glucose utilisation only result in small changes in circulating plasma concentration. The measurement of plasma glucose also has the disadvantage that increases in adrenal cortical activity, due to stress factors, can cause large,

transitory increases in plasma glucose concentration that do not reflect the energy status of the animal (Lindsay, 1978).

Plasma glucose is under a high degree of homeostatic control. However, if glucose requirement is high (eg for lactose formation in the udder in early lactation or for the rapidly developing foetus in late pregnancy) or if dietary factors lead to reduced supply of glucose then hypoglycaemia may result.

1.6.1.2 Non esterified fatty acids (NEFA)

It is well established that increased mobilisation of adipose tissue results in an elevated plasma concentration of free fatty acids or NEFA during periods of energy restriction in ruminants (Annison, 1960; Patterson *et al.* 1964). Everts (1990) observed concentrations of plasma NEFA as low as 0.07 mmol/l in a barren ewes and the highest level of 2.74 mmol/l in a ewe suffering from acetonaemia.

Plasma NEFA concentration increased with decreasing energy status in pregnant goats (Hussain *et al.*, 1996), and in non-pregnant, non-lactating beef cows (Russel and Wright, 1983). Similarly, Pethick *et al.* (1983) found that the fasting of pregnant ewes for 3 to 4 days caused a two-fold increase in arterial NEFA concentration. Petterson *et al.* (1994) compared well fed and under fed ewes, which were either pregnant or non-pregnant. They found that both pregnancy (at 129 to 136 days) and underfeeding significantly increased plasma NEFA concentrations. In a similar study, Symonds *et al.* (1989) looked at the effects of underfeeding and shearing on pregnant ewes and found that underfeeding and shearing both increased the concentration of plasma NEFA and concluded that the effects of shearing are due to the long term metabolic adaption to increased cold exposure. Hussain *et al.* (1996) reported that goats

fed a poor quality roughage had a significantly higher plasma NEFA concentration than those fed good quality roughage. Dunshea and Bell (1989), in a study of lactating goats, reported that plasma NEFA concentrations decreased as lactation advanced and that plasma NEFA concentrations in turn were positively related to estimated body fat loss. The direct relationship between the degree of undernutrition, the rate of fat mobilisation and the plasma NEFA concentration has lead some workers to use it as a index for moderate degrees of undernutrition (Russel and Wright, 1983) and others to use it to maintain ewes in certain nutritional states in late pregnancy, (Russel *et al.*, 1967a).

Dietary protein level has also been shown to influence plasma NEFA concentration, for example, Cowan *et al.* (1981) found that lactating ewes on a high protein diet had an elevated plasma NEFA concentration compared with ewes on a low protein diet (0.43 mmol/l +/-0.09 and 0.31 mmol/l +/-0.06 respectively). In this instance, ewes on the high protein diet were mobilizing increased amounts of body fat to support milk yields nearer to their genetic potential. In suckler cows, Sinclair *et al.* (1994) reported that the provision of diets that were high in DUP compared to diets low in DUP resulted in increased plasma NEFA concentrations when cows were in negative energy balance and lower plasma NEFA concentrations when they were fed close to their energy requirements.

One of the potential problems with the use of plasma NEFA as an indicator of the rate of body fat loss is the potential for the rate of NEFA use by body tissues to vary and therefore make circulating plasma NEFA levels difficult to interpret. Cowan *et al.* (1981) in an experiment with lactating ewes, found that although relatively high rates of body fat mobilisation were measured, the plasma NEFA concentrations were low. This problem has lead many workers to measure NEFA entry rates. Dunshea and Bell (1988) found a highly significant correlation

between whole body NEFA entry rate, plasma NEFA concentrations and estimated body fat loss. This would imply that the measurement of plasma NEFA is a suitable indicator of the rate of body fat loss in ruminants. Plasma NEFA concentrations also increase rapidly in response to adrenaline secretions (Kronfeld, 1965; Bowden, 1971) and therefore potential problems with the use of this index exist with animals which are likely to be become disturbed during the collection of blood. Plasma NEFA is a very sensitive index of moderate undernutrition, but is less useful in more severe cases, probably reaching an upper limit (Russel *et al.*, 1967a).

1.6.1.3 β-hydroxybutyrate (BHB)

In the ruminant, BHB is derived either from conversion of butyrate produced in the rumen (Britton and Krehbiel, 1993) or through ketone body production when fatty acids are oxidised in the liver to produce acetyl-CoA and BHB, leading to its use as an indicator of energy intake (Russel *et al.*, 1967a). Low concentrations of plasma BHB have been observed in non-pregnant ewes (0.18 mmol/l), whilst higher values have been seen in ewes suffering from acetonaemia (8.81 mmol/l; Everts, 1990). The plasma ketone BHB only shows small increases in concentration during moderate undernutrition, but is more responsive in cases of severe undernutrition when plasma NEFA is less useful (Russel, 1979).

Hussain *et al.* (1996) reported increases in plasma ketone concentration due to sub-maintenance feeding of goats in late pregnancy. In another experiment, O'Doherty and Crosby (1998) also found that ewes subjected to energy deficits on days 121, 128, 135 and 142 of pregnancy had higher plasma BHB concentrations. Increases in plasma BHB concentration in pregnant ewes, due to underfeeding are much greater when ewes are shorn compared to unshorn due to increases in energy requirement (Symonds *et al.*, 1989). Everts (1990) also showed that plasma BHB concentrations taken from late pregnant ewes increase rapidly with

increasing litter size, whilst Hussain *et al.* (1996) reported higher plasma ketone concentrations in late pregnant goats fed poor quality silage, compared to those fed hay or good quality silage, an effect probably due to the higher butyric acid content of poor quality silage. In lactation, similar responses were found in cattle by Drackley *et al.* (1991), who reported that reducing *ad libitum* intake by 20% from days 14 to 42 of lactation in multiparous Holstein cows resulted in transient increases in plasma BHB concentrations.

Plasma ketone levels are also elevated during periods of low blood glucose, due to an increase in hepatic ketone formation (Hove and Halse, 1983). Lack of available glucose can reduce the production of oxaloacetate, a key metabolite in the TCA cycle. This, in turn, inhibits the use of the TCA cycle for the oxidation of mobilised fatty acids, resulting in a build up of ketones during prolonged periods of adipose tissue mobilisation (Payne,1989).

Russel (1984) deemed that BHB is the most suitable indicator of energy status in a wide range of situations. It has been used as an indication of acceptable levels of energy status in pregnant ewes (Russel, 1977) and pregnant cows (Russel and Wright, 1983). BHB is less subject to change from extraneous factors associated with the handling and blood sampling of animals which are not accustomed to these procedures (Russel, 1984). The usefulness of BHB as an indicator of energy status lead Russel (1984) to produce empirical equations to relate plasma BHB concentration to energy status of the pregnant ewe. Russel, (1984) concluded that for an individual ewe a plasma BHB concentration of 1.1 mmol/l would constitute a satisfactory nutritional state, but due to the inevitable natural variation within the flock situation, a mean plasma concentration of 1.1 mmol/l would lead to some unacceptably high plasma BHB concentrations and therefore a mean value of 0.8 mmol/1 should be adopted.

1.6.2 Indicators of protein status

The principle blood parameters that can be used as indices of protein status in ruminants are; plasma urea-nitrogen, albumin, total protein and globulin (Topps and Thompson, 1984).

1.6.2.1 Urea-nitrogen

The concentration of plasma urea-N is a useful tool for estimating the protein status of ruminants (Topps and Thompson, 1984). Plasma urea-N concentration is a measure of the surplus supply of ammonia, arising from both digestion and metabolism which goes into the blood stream and is converted to urea by the liver and its concentration is related to the level of dietary protein intake (Topps and Thompson, 1984). For example, McNeill *et al.* (1996) showed that ewes in late pregnancy fed a diet containing 157 gCP/kg DM had a significantly higher plasma urea-N concentration than ewes fed 79 gCP/kg DM (1.94 and 4.00 mmol/l respectively), whilst in wethers, Hatfield *et al.* (1998) reported that blood urea nitrogens were higher in those fed a 180 g/kg compared to a 100 g/kg CP diet. The majority of plasma urea is excreted via the kidneys, although a variable amount will be recycled to the rumen via saliva (Payne, 1989). High plasma urea-N concentration can also indicate a deficiency of FME relative to ERDP (Cannas *et al.*, 1998). Incorporation of rumen ammonia into microbial protein can be limited by the available energy which may lead to transient increases in plasma urea-N concentration (Witt *et al.*, 2000). Higher plasma urea-N values may also be observed when MP:ME intake is supra-optimal (Newbold, 1994). The animal may try to optimise this ratio by deaminating a proportion of amino acids, thus producing elevated plasma urea concentrations.

1.6.2.2 Albumin

The major function of albumin is the maintenance of colloid osmotic pressure in the blood (Payne, 1989). Plasma albumin concentrations are a useful indicator of the animals ability to synthesise and store protein, and low values may indicate insufficiency of protein and/or energy over an extended period (Manston *et al.*, 1975; Topps and Thompson, 1984). When low plasma albumin and urea-N concentrations are found together it is likely to be due to inadequate dietary intake of protein (Manston *et al.*, 1975; Topps and Thompson, 1984). However, if low plasma albumins and high urea-N concentrations are observed it is more likely to be due to a parasitic or pathogenic infection of the liver, reducing the animals ability to synthesise albumin and thus lowering plasma concentrations (Topps and Thompson, 1984). Plasma albumin and urea-N concentrations taken from ewes in the late gestation appear to reflect protein intake (Lynch and Jackson, 1983; Thomas *et al.*, 1988). O'Doherty and Crosby (1998) reported a significant reduction in plasma albumin concentration in ewes as pregnancy advanced from day 121 to day 142 of gestation (23.7 *v.* 20.9 g/l), reflecting an increased protein demand of the rapidly growing foetus and for the synthesis of colostrum. Similarly, Manston *et al.* (1975) reported that plasma albumin concentration decreased as lactation advanced in dairy cows on low protein diets, but not on high protein diets. Manston *et al.* (1975) postulated that the continued demand for pre-cursors for the production of milk resulted in a reduction in the synthesis of other proteins, such as albumins, when protein supply was limiting. Plasma albumin has a half life of about 30 days so that any decrease in synthesis does not usually result in reduced plasma concentrations for several weeks (Topps and Thompson, 1984; Payne, 1989). Low concentrations of plasma albumin are therefore more likely to indicate long, rather than short term protein deficiency.

1.6.2.3 Total Protein and Globulins

Total protein content of blood is made up of albumins and globulins (Topps and Thompson, 1984). Laboratory determination of globulin concentration in blood is traditionally calculated as the difference between the concentrations of total protein and albumin (Topps and Thompson, 1984). The value obtained for globulin concentration is therefore dependent on the accuracy of the value obtained for both total protein and albumin. Globulin also appears to vary inversely with albumin concentration and such a relationship would limit the changes in the osmotic pressure of the blood (Kitchenham and Rowlands, 1976). Changes in globulin concentrations may therefore be a secondary effect of the changes in albumin concentration (Rowlands, 1980). Baumgartner and Pernthaner (1994) reported that the normal range of plasma total protein is from 53 to 80 g/l in Karakul sheep. Lynch and Jackson (1983) found neither plasma total protein nor globulin concentration was sensitive to protein intake in late pregnant ewes. In the study of Lynch and Jackson (1983) ewes were fed diets containing either 120, 90, and 70 g/kg CP and had plasma total protein concentrations of 62, 63, 62 g/l respectively, whilst globulin concentrations were 31, 32, and 33 g/l respectively. Total protein and globulins may therefore be of limited use in the determination of short term protein status.

1.7 IMPROVING THE PROTEIN SUPPLY IN RUMINANTS

The principal objective of protecting proteins from rumen degradation is to enhance the supply of essential amino acids to the productive animal (Beever and Thomson, 1977; Ljøkjel *et al.*, 2000). This is achieved by reductions in rumen degradation and concurrent increases in the quantities of CP and amino acids entering and absorbed from the small intestine (Demjanec *et al.*, 1995). A summary of the principle methods of reducing rumen degradation of dietary proteins is provided in Table 1.14.

Table 1.14 *A summary of methods for reducing the rate and extent of protein degradation in the rumen*

Factor	Comments	Reference
Protein source	Insoluble proteins generally have a lower rate of degradation in the rumen.	1
Chemical treatment	Causes a reduction in protein solubility as cross linkages form between the various chemicals and amino or amide groups. Chemicals include formaldehyde, acetic acid, propionic acid, sodium hydroxide, acrolein acetals, hexamethylene-tetramine, phosphonitritic halides, polymerized unsaturated carboxylic acids, sulphonyl halides and acetylenic esters.	2,3
Tannins	Tannins are polyphenolic compounds of plant origin, which bind with proteins, primarily by hydrogen bonding.	4
Heat	Heat treatment causes a reduction in protein solubility via a Maillard reaction sequence between ε-amino groups of lysine residues and carbonyl compounds.	3,4,5
Encapsulation	Degradable protein can be encapsulated by less degradable material. Materials used include fat and whole blood.	6,7,8
Rumen outflow rate	Any factor which will decrease residence time in the rumen will decrease the effective protein degradability.	1,9
Inhibition of proteolysis	Reducing the numbers of proteolytic bacteria or inhibition of proteolytic enzymes.	4,10

1. Satter (1986).
2. Chalupa (1975).
3. Mustafa *et al.* (2000).
4. Broderick *et al.* (1991).
5. Moshtaghi Nia and Ingalls (1995).
6. Rossi *et al.* (1999).
7. Srivastava and Mani (1991).
8. Matsumoto *et al.* (1995).
9. Russell and Hespell (1981).
10. Wallace (1996a).

Of the methods listed in Table 1.13, heat and formaldehyde treatment are the most commonly used (Mustafa *et al.*, 2000).

1.7.1 Amino acid profile and availability in treated feedstuffs

As well as protein degradability and subsequent digestibility in the small intestine, the quality of the undegradable protein is also very important. A good quality protein would complement the amino acid profile of the bacterial protein leaving the rumen to match the requirements of the productive ruminant (Rodehutscord *et al.*, 1999). Although formaldehyde and heat treatment increases the availability of total amino acids and total essential amino acids available to the ruminant (Barry, 1976), the relative proportions may change (Moshtaghi Nia and Ingalls, 1995; Mustafa *et al.*, 2000).

1.7.1.1 The effect of heat treatment on the amino acid composition

Undi *et al.* (1996) reported that moist heat treatment of canola meal (low glucosinolate rapeseed meal) did not drastically alter the essential amino acid composition with the exception of lysine, tryptophan and arginine which were all reduced (Table 1.15). Comparable results have been found by other authors. Moshtaghi Nia and Ingalls (1995) reported that lysine concentration was reduced by 15.9 and 29.2% and arginine concentration reduced by 8.0 and 15.2% when canola meal was heated for 15 or 45 minutes respectively. In addition, Dakowski *et al.* (1996) reported that an increase in temperature during the treatment of rapeseed meal from 117 to 154°C caused total lysine concentration to decrease from 23 to 17 mg/g DM. Similar results are apparent for soya-bean meal where heating in the presence of xylose reduced the concentrations of lysine and arginine by 17 and 7% respectively (Harstad and Prestløkken, 2000). Lysine is usually the most sensitive amino acid to heat and is often lost at a rate of 5-15 times greater than other amino acids (Dakowski *et al.*, 1996). Additionally, it is one of the first

limiting amino acids for milk synthesis (Schingoethe, 1996).

Table 1.15 *Essential amino acid composition (mg/g DM) of canola meal autoclaved at 127 ± 1°C with a steam, pressure of 117KPa for 0, 15, 45 or 90 minutes*

	Heat treatment (minutes)			
	0	15	45	90
Crude protein	418	414	416	420
Methionine	8.1	8.2	7.6	8.1
Lysine	19.6	17.3	15.2	12.6
Threonine	14.8	15.3	15.0	16.1
Leucine	23.5	24.8	24.5	27.2
Isoleucine	8.8	10.1	10.3	12.4
Tryptophan	3.4	3.1	2.7	2.1
Phenylalanine	13.3	13.8	13.7	15.0
Histidine	8.8	9.2	9.0	9.7
Arginine	20.0	20.0	18.0	16.7
Valine	11.9	13.8	13.9	16.6
Total amino acids	362	373	364	387

(From Undi *et al.*, 1996).

1.7.1.2 The effect of formaldehyde treatment on the amino acid composition

Tyrosine availability is often reduced in formaldehyde treated feedstuffs due to the formaldehyde reacting with tyrosine by a so called electrophilic aromatic substitution resulting in the bonds formed being more difficult to split or to lyse (Antoniewicz *et al.*, 1992). Erfle *et al.* (1986) observed reductions in both the lysine and tyrosine content of formaldehyde treated soya-bean meal (Table 1.16). Treating soya-bean meal with 0 g/kg and 9 g/kg of formaldehyde reduced the content of lysine from 61.3 to 47.2 and tyrosine from 23.4 g/kg to 8.0 g/kg respectively (Erfle *et al.*, 1986).

Table 1.16 *Effect of additions of formaldehyde on the lysine and tyrosine content of soya-bean meal*

	Amino acid content (g kg^{-1} protein)	
Formaldehyde (g kg^{-1} soya-bean meal)	Lysine	Tyrosine
0	61.3	23.4
1	51.8	17.7
3	53.1	17.4
6	51.1	14.5
9	47.2	8.0
SE	1.77	1.69

(Adapted from Erfle *et al.*, 1986).

In addition to the effects of formaldehyde or heat treatment on the amino acid profile of the feed, it is possible that a reduction in the total amino acids absorbed at the small intestine may occur as a result of reductions in rumen protein degradability with concurrent reductions in microbial protein synthesis (Beever *et al,* 1976) because of the over protection of the protein in the small intestine, rendering the protein unsusceptible to mammalian enzymes (Varvikko, *et al.*, 1983; Antoniewicz *et al.*, 1992). Reductions in bacterial N flows at the duodenum were observed when wethers were fed diets containing heat treated soya-bean meal (Demjanec *et al.*, 1995). The likely cause of this was reported as a reduction is rumen degradable protein supply to an extent where the ERDP:FME supply was sub-optimal (Demjanec *et al.*, 1995). This effect could equally occur when feeding protein which confers some natural protection from degradation (e.g. fishmeal and blood meal) or when using other methods of protection (e.g. blood treatment). Other studies have also observed similar effects of reducing dietary protein degradability on microbial protein synthesis, even when degradable protein supply (measured by rumen ammonia concentrations) have been maintained at levels believed to be in excess of microbial requirements (Cecava *et al.*, 1990 and 1991). This may be attributed to the growth of some species of rumen bacteria being limited by an inadequate supply of free amino acids, branched chained amino acids or small peptides (Demjanec *et al.*, 1995).

1.7.2 Rumen degradability of nitrogen

1.7.2.1 Heat treatment

Reductions in the rumen degradability by the heat treatment of vegetable proteins is caused by the Maillard reaction sequence between ε-amino groups of lysine residues and carbonyl compounds. Methods of heat treatment include; dry heat treatment, moist heat treatment (autoclave) and micronisation (Mustafa *et al.*, 2000). Heat is often used in the preparation of feedstuffs for ruminants, particularly during pelleting and reduces rumen protein degradability (Subuh *et al.*, 1994; Demjanec *et al.*, 1995; Dakowski *et al.*, 1996; Rodehutscord *et al.*, 1999), increasing rumen escape protein (Demjanec *et al.*, 1995). Heat is one of the most common methods of protection used (Mustafa *et al.*, 2000).

It is apparent from the results presented in Table 1.17 that the reduction in degradability is affected by both temperature and duration of heating. However, the data presented confirms the comments made by Mir *et al.* (1984) that temperature has a greater effect on degradability than the duration of heating. This effect is illustrated in Table 1.17 by the work of Subuh *et al.* (1994) and Dakowski *et al.* (1996). For example, Subuh *et al.* (1994) found that heating rapeseed meal at a relatively low temperature (110°C) for an extended period (120 minutes) only caused a 3% reduction in rumen degradability, whilst Dakowski *et al.* (1996) found a 79% reduction in the degradability for the same protein type after heating for only five minutes at the higher temperature of 150°C.

Table 1.17 *The effects of temperature and duration of heating soya-bean meal *, rapeseed meal # and sunflower oilcake ▼ on the percentage reduction in rumen nitrogen degradability compared to the un-heated control*

	Duration (minutes)						
Temperature (°C)	0	5	10	30	60	90	120
0	0						
100					$0^{\#LI}$		
110							$3^{*S}/3^{\#S}$
120				21^{*L}			
130		$24^{\#D}$		48^{*L}	$36^{\blacktriangledown SC}$	$44^{\blacktriangledown SC}$	$48^{\blacktriangledown SC}$
140		$70^{\#D}$					
150		$79^{\#D}$		$53^{\blacktriangledown SC}$	$58^{\blacktriangledown SC}$		
170			$44^{\blacktriangledown SC}$				
200					$52^{\#LI}$		

(Data sources presented by: [S] Subuh *et al,*. 1994; [L] Ljøkjel *et al.*, 2000; [D] Dakowski *et al.*, 1996; [LI] Lindberg *et al.*, 1982 and [SC] Schroeder *et al.*, 1996).

1.7.2.2 Formaldehyde treatment

Formaldehyde treatment reduces rumen nitrogen degradability (Varvikko *et al.*, 1983; Setala and Syrjala-Qvist, 1984; Antoniewicz *et al.*, 1992; Hadjipanayiotou, 1992; Subuh *et al.*, 1994; Hadjipanayiotou and Photiou, 1995 and Rodehutscord *et al.*, 1999) and improves the calculated MP supply to the ruminant (Rodehutscord *et al.*, 1999). It is the most common chemical treatment used to reduce rumen degradation (Mustafa *et al.*, 2000). Similar to other protection methods, formaldehyde reduces rumen nitrogen (N) degradation of dietary protein by reducing the rapidly soluble N fraction and the rate of N degradation of the slowly degradable fraction (Mustafa *et al.*, 2000).

Formaldehyde reacts with the terminal amino, the ε-amino group of lysine, the primary amide groups of asparagine and glutamine, the guanidyl group of arginine, the hydroxyl groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group of phenylalanine, the indole group of tryptophan and the imadazole group of histidine

(Barry, 1976). Condensation reactions then take place slowly over time, with the formation of stable methylene cross-linkages between protein chains (Barry, 1976). Most of the reactions caused by the action of formaldehyde should be reversible under the actions of the more acid solutions found in the small intestine and should therefore not affect post ruminal protein digestibility (Antoniewicz *et al.*, 1992). However, irreversible cross linkages are possible due to overprotection (Varvikko, *et al.*, 1983; Mir *et al.*, 1984; Antoniewicz *et al.*, 1992).

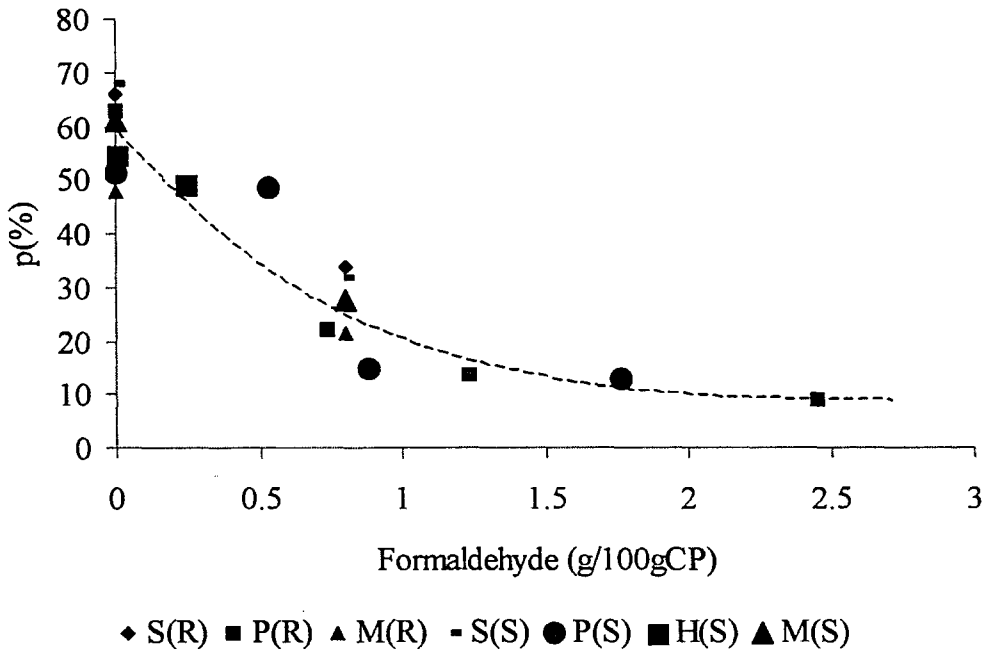

Figure 1.6 The effect of formaldehyde treatment on the effective ruminal crude protein (CP) degradability (p) of soya-bean meal (S) and rapeseed meal (R), (Data sources presented by: S = Subuh *et al.*, 1994; P = Park *et al.*, 1999; M = Mir *et al.*, 1984; H Hadjipanayiotou, 1992).

It is apparent from Figure 1.6 that formaldehyde treatment reduces the ruminal CP degradability of rapeseed meal and soya-bean meal. The magnitude of the reduction increases with the amount of formaldehyde applied. Initial additions of formaldehyde caused large reductions in rumen CP degradability, with further additions beyond about 0.8 g/100 g CP having minimal effect.

1.7.3 Digestibility of nitrogen in the small intestine

1.7.3.1 Heat treatment

Heating for extended periods of time and at high temperatures has detrimental effects on the intestinal digestibility of UDP (Table 1.18). For example, Schroeder *et al.* (1996) presented data to show that heating sunflower oil cake for 60 minutes at 130°C reduced the digestibility by 3.3%, whilst heating for extended periods (120 minutes, 130°C) and at higher temperatures (10 minutes, 170°C) further reduced the intestinal digestibility (5.2 and 10.8% respectively; Table 1.18).

Table 1.18 *The effects of temperature and duration of heating soya-bean meal* [*], *rapeseed meal* [#] *and sunflower oilcake* [▼] *on the percentage change in intestinal digestibility of samples pre-incubated in the rumen for 16 hours*

Duration	Temperature (°C)				
(minutes)	0	120	130	150	170
0	0				
5			+11.0$^{\#D}$	0.0$^{\#D}$	
10					-10.8$^{\blacktriangledown SC}$
30		+0.5^{*L}	0.0^{*L}	-11.0$^{\blacktriangledown SC}$	
60			-3.3$^{\blacktriangledown SC}$		
90			-5.2$^{\blacktriangledown SC}$		
120			-6.7$^{\blacktriangledown SC}$		

(Data sources presented by: [L] Ljøkjel *et al.*, 2000; [D] Dakowski *et al.*, 1996; and [SC] Schroeder *et al.*, 1996).

However, Schroeder *et al.* (1996) also reported that the effects of heating caused proportional increases in the UDP that were greater than the reduction in digestibility of UDP in the small intestine, leading to increases in the digestible UDP as a proportion of CP (Table 1.19).

Table 1.19 *The effect of duration of heating and temperature on digestibility of undegraded dietary protein (UDP; %), proportion of digestible UDP (g UDP/100 g CP) and acid detergent insoluble nitrogen (ADIN) concentration in UDP (%) of un-heated and heat processed sunflower oilcake*

Temperature (°C)	Duration (minutes)	Digestibility of UDP (%)	Digestible UDP (g per 100g CP)	ADIN (%)
0	0	96.2	10.9	2.39
130	60	93.0	43.2	6.33
130	90	91.2	48.6	9.27
130	120	89.8	49.7	12.04
150	30	85.6	51.5	14.40
150	60	78.3	49.3	18.16
170	10	85.8	45.1	10.31

(From Schroeder *et al.*, 1996)

The data presented in Table 1.19 also demonstrate that the reduction in intestinal digestibility at the higher temperatures is reflected in the higher ADIN concentrations measured (2.39% for the untreated and 10.31% for the sunflower cake heated at 170°C for 10 minutes respectively). This observation is in agreement with Dakowski *et al.* (1996) who found that the proportion of ADIN in total N increased from 5.5 to 17.4% when previously untreated rapeseed meal samples were heated at 150°C for 5 minutes.

To define optimum conditions for heat-treating proteins it is necessary to quantify the disappearance of CP within the different sections of the intestinal tract (Table 1.20). Heating rapeseed meal to 125°C for up to 30 minutes increased the amount of dietary protein available for digestion in the small intestine without affecting total tract digestibility of CP. However, heating to 145°C resulted in heat-damaged protein with lower intestinal and total tract digestibility. It can be concluded from the above study that heating rapeseed meal to 125°C for 10 to 30 minutes is a viable method of reducing ruminal degradability without compromising the intestinal or total tract digestibility of UDP.

Table 1.20 *The effect of dry heat treatment on ruminal, intestinal and total tract disappearance of crude protein of low glucosinolate rapeseed meal samples incubated in the rumen for 12 hours*

	Control	125°C			145°C			SEM
		Duration (minutes)						
	0	10	20	30	10	20	30	
CP disappearance (%)								
Rumen	64.3	33.8	31.0	30.6	24.6	24.9	19.7	1.1
Intestinal[2]	80.5	85.5	83.0	84.2	54.4	59.3	49.0	3.5
Total tract	93.0	90.4	88.3	89.0	64.4	69.4	57.9	2.7

(Adapted from McKinnon *et al.*, 1995) [2] Percentage of rumen undegraded crude protein.

1.7.3.2 Formaldehyde treatment

There is a lack of information on the effects of formaldehyde treatment on intestinal digestibility of CP. However, Antoniewicz *et al.* (1992) reported that treatment with 1 or 2 g formaldehyde per 100 g CP was effective at reducing the rumen CP degradability of lupins, peas, rapeseed meal and field beans and the decline in rumen degradability was nearly completely compensated for by increases in intestinal digestion. However, in the same study, intestinal CP digestibility of rapeseed meal treated with 3 or 4 g formaldehyde per 100 g CP was highly reduced, lowering total tract CP disappearance (Figure 1.7).

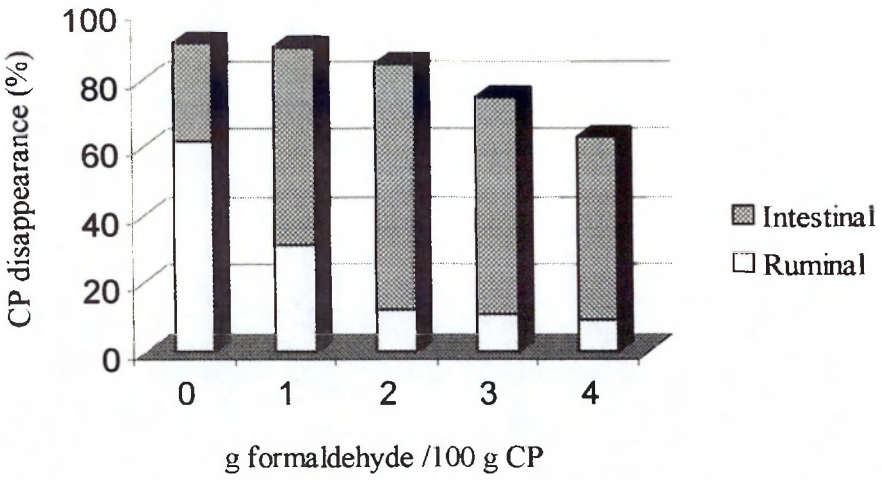

Figure 1.7 Ruminal and intestinal digestibility of crude protein (% of original content) of rapeseed meal treated with varying levels of formaldehyde, estimated by a 12 h ruminal *in situ* incubation and by the mobile bag technique, respectively (From Antoniewicz *et al.*, 1992).

Phillips *et al.* (1981) suggested that the optimum level of formaldehyde treatment of soya-bean meal was between 0.7 and 1.0 g formaldehyde per 100 g CP whereas Spears *et al.* (1985) suggested that 0.6 g formaldehyde per 100 g CP was the most effective at lowering rumen degradation, without reducing post-rumen digestion. Barry (1976) demonstrated that the quantities of total and total essential amino acids (g/d) leaving the abomasum were markedly increased by formaldehyde treatment of casein (1.2 g formaldehyde / 100 g CP) and groundnut meal (0.9 g formaldehyde / 100 g CP), with the increase being greater for the more soluble casein and that formaldehyde treatment increased the quantities of total amino acids and total essential amino acids (g/d) absorbed from the intestine.

1.7.4 Protecting amino acids from rumen degradation

Diets for monogastric animals of agricultural importance have routinely been fortified with the amino acids methionine and/or lysine (Sloan, 1997). However, although it has been recognised for some time that ruminants could benefit from supplementation with amino acids, direct fortification of diets has been deemed impractical because rumen microbes easily hydrolyse

these amino acids before they reach the small intestine (Clark, 1975) and that some method of rumen protection was required.

In designing a method which will confer a level of protection to supplemented amino acids, Wu and Papas (1997) defined the important criteria for amino acid protection as; efficacy (ideally the method should give amino acids total protection from rumen fermentation and allow complete release and absorption in the small intestine), safety (the components used to protect amino acids from ruminal degradation must be safe for animals and humans and must not generate undesirable residues) and cost (should be cheaper than the traditional approach of supplementing with protein sources which supply suitable amounts of the amino acids to the small intestine). Efforts to protect amino acids for ruminants have concentrated on methionine, lysine, threonine and tryptophan as these are often found to be first limiting for growth, lactation or wool growth (Nimrick *et al.*, 1970).

1.7.4.1 Heat and formaldehyde treatment

Heat and formaldehyde treatment is often used for the ruminal protection of intact vegetable proteins (Antoniewicz *et al.*, 1992; Hadjipanayiotou, 1992; Subuh *et al.*, 1994; Hadjipanayiotou and Photiou, 1995; Rodehutscord *et al.*, 1999). By treating proteins rich in limiting amino acids it may be possible to enhance the relative value of the protein at the absorption sites (Undi *et al.*, 1996). However, predictability and control of rumen protection is poor and the post-ruminal release of amino acids is generally inversely related to the degree of ruminal protection (Antoniewicz *et al.*, 1992). In addition, heat and formaldehyde has been shown to reduce the concentration of lysine and sulphur containing amino acids in proteins (Moshtaghi Nia and Ingalls, 1995; Rodehutscord *et al.*, 1999). These methods are unsuitable for the protection of these individual amino acids (Wu and Papas, 1997).

1.7.4.2 Low solubility analogues

Low solubility peptides (eg. peptides of lysine) and amino acid derivatives (eg. derivatives of methionine) have been evaluated as sources of protected amino acids for ruminants (Wu and Papas, 1997). However, these have had limited success because of poor post-ruminal bioavailability (lysine peptides) or minimal reduction in ruminal degradability (methionine derivatives) (Papas *et al.*, 1974).

1.7.4.3 Lipid based formulations

Lipid protection of amino acids relies on the inherent resistance of fatty acids to enzymes in the rumen to maintain the integrity of the protective coating. Subsequent digestion by enzymes in the small intestine, releases the amino acid(s) for absorption (Rossi *et al.*, 1999). Like other methods of protection a balance must be reached between reduction in rumen degradation and subsequent availability in the small intestine. Amino acids can either be embedded in the lipid complex or are formulated into small spheres and coated with lipid (Wu and Papas, 1997). The literature suggests that coating amino acids with lipid appears to be superior to embedding in a lipid complex as it will allow the lipid to carry a greater payload and is more likely to yield increased amounts of available amino acid in the small intestine (Casper *et al.*, 1987). Commercial fat coated products containing 85% DL-methionine have been reported to give 85% protection after 5-6 hours of rumen incubation (Wu and Papas, 1997).

1.7.4.4 pH-sensitive polymeric coatings

The use of a ruminally inert, pH-sensitive polymer has been used to protect amino acids in the rumen and release them in the small intestine (Baldwin *et al.*, 1993). The development of the technology relies on the difference in pH between the rumen and the abomasum (pH 5.5-7.0 and 2.0-3.0 respectively).

In early polymer coating systems cellulose propionate 3-morpholinobutyrate was used as an acid soluble polymer for coating amino acids such as methionine and lysine. Following this initial work a more effective delivery system was developed which used co-polymers of vinyl pyridine and styrene (Wu and Papas, 1997). The coating system is composed of a basic polymer such as a poly (2-vinylpyridine-co-styrene, 80:20), a pigment material such as talc or aluminium and a hydrophobic substance such as steric acid at typical percentage inclusions of 31.5 : 63.5 : 5.0 by weight. This later system was named the reverse-enteric coating system (Wu and Papas, 1997). These authors reported that polymer coating weights of around 12% cause the maximum reduction in rumen degradation of methionine without reducing availability in the small intestine. An acidic medium generates pinholes immediately on the coating surfaces, thus causing the release of amino acid through the irreversible rupture of the coating (Wu and Papas 1997). Smartamine™ M (Rhône Poulenc) which is presented as 2mm beadlets and contains 70% DL-methionine is produced by the pH sensitive, reverse-enteric coating system described.

1.7.5 Animal performance

1.7.5.1 Performance of animals fed formaldehyde treated protein sources

The literature reveals a number of experiments designed to investigate the possible production benefits of including formaldehyde treated protein sources in ruminant rations, but the conclusions drawn are not always consistent. Tewatia *et al.* (1995) fed lactating goats 400 g per day of faba beans that were either untreated or treated with 0.43 or 0.54 g of formaldehyde per 100 g CP. Treatment with either level of formaldehyde had no effect on the total yield of milk or on the concentration of milk fat and total solids, but total milk protein content was significantly lower in milk from goats that were fed formaldehyde treated faba beans. Milk protein contents were 51.3, 48.6 and 45.3 g/l for goats fed faba beans that were untreated or

treated with 0.43 and 0.54 g formaldehyde per 100 g CP respectively (Tewatia *et al.*, 1995). Hadjipanayiotou (1992) also observed no difference in the total yield of milk, fat or total solids in lactating goats that were fed concentrates including untreated soya-bean meal or soya-bean meal treated with 0.24 g of formaldehyde per 100 g of soya-bean CP . Studies with dairy cows have also shown variable results when vegetable protein sources are treated with formaldehyde. Erfle *et al.* (1986) treated soya-bean meal with 0.3 g of formaldehyde per 100 g CP and found no improvements in the yield of milk or fat in dairy cows, but found a significant reduction in protein yield. Erfle *et al.* (1986) concluded that the lack of a response may have been due to the reduced concentrations of rumen ammonia observed, causing reductions in microbial populations. Alternatively, an observed reduction in the concentrations of lysine and tyrosine in formaldehyde treated soya-bean meal could account for the reduction in yield, particularly as lysine is considered one of the first limiting amino acids for milk production in high yielding dairy cows (Clark, 1975). In contrast to the above reports, Hamilton *et al.* (1992) reported higher yields of milk and protein from cows at pasture fed sunflower meal treated with 0.5 g of formaldehyde per 100 g CP and 0.7 g of formaldehyde per 100 g CP compared to those fed the same amount of untreated sunflower meal.

1.7.5.2 Production responses to protected amino acids

There is a lack of published information on production responses to supplementing pregnant and lactating ewes with rumen-protected amino acids. However, for late pregnant ewes, Robinson (1990a) noted that microbial protein was likely to be deficient in cystine and the formation of cystine from methionine may also cause methionine to become limiting. Late pregnant ewes may therefore benefit from supplements of rumen-protected methionine, particularly when the main source of digestible undegradable protein, soya-bean meal for example, is also low in methionine (Rossi *et al.*, 1999). In lactation, Lynch *et al.* (1991)

observed increased growth rates of lambs suckled by ewes fed rumen-protected methionine and lysine and concluded that the growth of young suckling lambs may respond to an optimal intake of specific dietary amino acids. Sevi *et al.* (1998) also found beneficial effects of feeding rumen-protected methionine and lysine to lactating ewes. Ewes receiving the amino acid supplemented diet had a higher yield of milk, milk protein, milk fat and milk lactose and had a higher gross efficiency of utilisation of dietary nitrogen (Sevi *et al.*, 1998). In contrast to the results of Lynch *et al.* (1991) and Sevi *et al.* (1998), Baldwin *et al.* (1993) found no effect of feeding rumen protected methionine on ewe bodyweight change, milk production and subsequent lamb growth in Dorset ewes. The lack of any response in the experiment of Baldwin *et al.* (1993) could have been due to another amino acid (eg lysine) being first or co-limiting to milk production, or possibly due to insufficient ruminal protection. Rumen protected methionine has also been shown to increase clean wool growth in growing wethers (Rodehutscord *et al.*, 1999; White *et al.*, 2000) and mature ewes (Cottle, 1988). Rodehutscord *et al.* (1999) and White *et al.* (2000) reported increases in body weight gain in Merino wethers fed protected methionine of 27 and 22% respectively. Reductions in urinary N excretion and increases in N retention have also been recorded in cashmere-yielding and Angora goats fed diets supplemented with 2.5 g/kg of rumen-protected methionine (Souri *et al.*, 1998).

1.7.5.3. Amino acid supplementation of formaldehyde treated protein sources

Rodehutscord *et al.* (1999) reported that there may be an increased requirement for certain rumen protected amino acids when the main protein fed has been formaldehyde treated. Rumen protected methionine supplementation improved wool growth in Merino wethers fed 350 g lupins per day by 19%, 37% and 36% when the lupins were fed untreated, heat treated or formaldehyde treated respectively (Rodehutscord *et al.*, 1999). Sulphur containing amino acids (SAA) are often first limiting for wool growth in sheep (Cottle, 1988) and Rodehutscord *et al.*

(1999) concluded that treatment of lupins with heat (115°C for 1 hour) or with approximately 11.5 g formaldehyde/kg of lupins reduced the availability of SAA. In the same experiment the body weight gain of wethers was improved by the addition of rumen protected methionine to diets, indicating that SAA may limit protein deposition. Improvements in wool growth and weight gain of wethers supplemented with methionine can be attributed to improvements in MP utilisation (Table 1.21).

Table 1.21 *The effect of feeding growing Merino wether sheep either untreated, heat treated or formaldehyde treated lupins with or without additions of rumen-protected methionine on the MP supply, wool growth, weight gain, N accretion in wool and the N accretion in weight gain (g/day)*

Lupin treatment	None		Heat		Formaldehyde	
Methionine	-	+	-	+	-	+
MP supply (g/day)	44	44	46	46	49	49
Wool growth (g/day)	60	73	56	71	51	69
Weight gain (g/day)	8.5	10.0	8.7	10.4	8.3	11.1
N accretion (g/day):-						
In weight gain	1.3	1.5	1.2	1.5	1.1	1.4
In wool	1.5	1.7	1.5	1.8	1.5	1.9
Total	2.8	3.2	2.7	3.3	2.6	3.3
N accretion/N supply from MP	0.40	0.45	0.37	0.45	0.33	0.42

(From Rodehutscord *et al.*, 1999).

1.8 CONCLUSIONS

Fishmeal has been shown to be a suitable source of UDP for ewes in late pregnancy and early lactation (Robinson, 1983a) and there is a lack of published information on alternative vegetable sources. Whilst formaldehyde treatment of vegetable protein sources has been shown to decrease the rumen degradability of protein and increase the supply of UDP (Mustafa *et al.*, 2000), animal production responses are often variable and little work has been published on the efficacy of their use as an alternative to fishmeal on the performance and metabolism of ewes in late pregnancy and early lactation.

In addition, recent advances in methods of ruminant diet formulation has allowed better prediction of both the rumen and post rumen supply of dietary protein (e.g. AFRC, 1993). This has enabled greater accuracy in comparing diets containing alternative protein sources that are either similar in all aspects of protein supply or which differ in only one aspect (eg increased DUP) on the performance of ewes in late pregnancy and lactation.

The objective of the experiments reported in this thesis were to test the null hypothesis that diets formulated to vary only in their source of protein will have no effect on metabolism and performance of twin-bearing ewes during late pregnancy and early lactation.

CHAPTER 2. MATERIAL AND METHODS

2.1 EXPERIMENTAL ANIMALS

The College flock of 160 Charollais x Lleyn, Friesland x Lleyn, Charollais x Cambridge and Suffolk x North of England Mule ewes were oestrus synchronised during the summers of 1997, 1998 and 1999, using progesterone impregnated sponges (Chronogest; Intervet Ltd, Holland), timed to cause lambing in early December each year. At the point of sponge removal ewes were administered 250 iu of pregnant mare serum gonadotrophin (PMSG) as an intramuscular injection. During their first oestrus after removal of the sponges, the ewes were artificially inseminated using Suffolk (experiment 1; Chapter 3) or Charollais sires (experiments 2 and 3; Chapters 4 and 5). Immediately after insemination Charollais rams (all experiments) were turned into the ewes. During the second oestrus Charollais rams (all experiments) were turned into the ewes after having a raddle crayon attached to the chest area to allow identification of ewes which had been mated. When ewes which conceived to the first oestrus reached 84 days of gestation, all ewes were pregnancy scanned using ultrasound to determine the number of foetuses carried.

2.2 DATA AND SAMPLE COLLECTION

2.2.1 Ewe liveweight and body condition score

Ewe liveweight and body condition score was measured at the same tome each week (Wednesday, 2pm). Liveweight was recorded using an EziWeigh 1 scales (Tru-Test Ltd, Auckland, New Zealand), whilst body condition was assessed on a scale of 1 (thin) to 5 (fat) according to the system described by Russel *et al.* (1969). The assessment of body condition was carried out by the same person over the duration of all experiments.

2.2.2 Lamb sire breed, birth weight and weekly weights

In the experiment reported in Chapter 3, where both Charollais and Suffolk sires were used, lamb sire breed was determined by face colour and leg colour. Lambs with a Suffolk sire were identified by their black face and legs, whilst lambs with a Charollais sire were identified by their light brown face and legs. In the experiments reported in Chapters 4 and 5, only Charollais sires were used.

Lamb birthweight was recorded (FG-60K scales; A and D Co., Ltd, Japan). Lambs were weighed after they had been licked by their mother, but prior to receiving any colostrum. On average this was about 20 minutes *post partum*. Lambs were also weighed at approximately 2 pm when they reached 7, 14, 21 and 28 days of age in all experiments reported. In addition, lambs in the experiment reported in Chapter 3 were also weighed when they reached 35 and 42 days of age.

2.2.3 Ewe colostrum production

The yield and quality of colostrum was assessed by the following method adapted from Pattinson *et al.* (1995).

After parturition lambs were prevented from obtaining colostrum from the ewe by use of an udder cover. Within one hour of birth ewes were given an intramuscular injection of 1 ml of oxytocin (LEO, Animal Health Division, Bucks) and both sides of the udder were milked out completely by hand. The quantity of colostrum obtained was measured using a 1000 ml measuring cylinder and after mixing colostrum from both sides of the udder, two 50 ml samples were taken and stored at -20°C prior to analysis. Excess colostrum was then fed back to the lambs at a rate of approximately 35 g/kg lamb birth weight.

Twelve hours after the end of the initial milking lambs were removed from their dam and placed behind a wire mesh barrier to prevent the lamb from sucking, but still maintaining visual and olfactory contact with the ewe. After administration of a 1 ml injection of oxytocin intramuscularly, the ewe was milked out fully and the time at the end of milking was noted. After allowing approximately four hours to elapse the ewe was given another 1 ml of oxytocin intramuscularly and milked out again. The quantity of colostrum was recorded and after thorough mixing of colostrum from both sides of the udder two 50 ml samples of colostrum were taken and stored at -20°C. Excess colostrum was offered to the lambs from a bottle and the lambs returned to the ewe. The actual time at the end of both the 12 hour milking and the subsequent milking was recorded to allow an accurate calculation of an hourly secretion rate of colostrum between 12 and 16 hours *post partum*.

Samples of colostrum were analysed for CP, fat, DM, ash, IgG and lactose content.

The 24 hour yield (g) was calculated as:

$$\text{Initial yield (g)} + \left\{ \frac{\text{16 hour yield (g)}}{\text{time elapsed between end of 12 hour milking and end of 16 hour milking (hours)}} \right\} \times 23$$

The 24 hour constituent yield (g) was calculated as:

$$\text{Initial yield (kg) x constituent concentration (g/kg)} + \left\{ \frac{\text{16 hour yield (kg) x constituent concentration (g/kg)}}{\text{time elapsed between end of 12 hour milking and end of 16 hour milking (hours)}} \right\} \times 23$$

2.2.4 Ewe milk production

In the experiments reported in Chapters 4 and 5, on days 7 and 21 days *post partum*, ewe milk production was measured by a method developed from Doney *et al.* (1979). At 10 am lambs were separated from the ewe by means of a mesh gate that allowed visual and olfactory contact to be maintained. Ewes were given a 1ml, intra-muscular injection of oxytocin and milked out completely and the time at the end of milking was noted. Approximately 4 hours later the ewe was given another intramuscular injection of oxytocin (1 ml) and milked out fully by hand. The time at the end of milking was recorded. After mixing, two 50 ml samples of milk were taken and frozen at -20°C until subsequent analysis was carried out. The lambs were returned to the ewe.

Samples of ewes milk were analysed for total solids, fat, CP and lactose (section 2.3.12).

Milk yield (g/h) was calculated as:

$$\left\{ \frac{\text{4 hour yield (g)}}{\text{time elapsed between end of first milking and end of second milking (hours)}} \right\}$$

Milk constituent yield (g/h) was calculated as:

$$\left\{ \frac{\text{4 hour yield (kg) x constituent concentration (g/kg)}}{\text{time elapsed between end of first milking and end of second milking (hours)}} \right\}$$

2.2.5 Ewe blood plasma

Blood samples were taken from individually penned ewes by jugular venepuncture into 7ml evacuated tubes (Vacutainer) containing the anticoagulants lithium heparin and potassium oxalate at times indicated in the respective experimental chapters. After sampling, the blood from each tube was immediately centrifuged for 15 minutes (Beckman Avanti 30 centrifuge) at 3000 rpm (1610 x g). Plasma was removed and put into 2 x 1 ml aliquots and stored at -80°C prior to subsequent analysis.

2.2.6 The *in situ* rumen degradability of nitrogen in concentrate feeds

2.2.6.1 Sample preparation and incubation

Nitrogen degradability coefficients of the concentrate feeds were determined by the method described by AFRC (1992). Samples of the diets were ground through a 2.5 mm screen and the small particles, less than 45 μm, removed by hand sieving. Approximately 5.0 g of dry matter (DM) of each concentrate was accurately weighed into synthetic fibre bags with a pore size of 43 μm^2 and a thread diameter of 40 μm. The bag, together with the sample, was then weighed. To allow suspension and retrieval of the bag and its contents from the rumen, a 25 cm length of nylon string was tied at one end around the mouth of the bag which was then securely closed by use of a rubber band. A maximum of 6 bags were placed into the rumen of a sheep at 0800 hours and incubated for 0, 2, 4, 8, 12, 24 and 48 hours. Sufficient bags were incubated to provide a pooled residue of approximately 7 g DM for each incubation time for each sheep.

2.2.6.2 Post incubation

After removal from the rumen the bags were washed through a domestic washing machine set on a 45 minute cold rinse cycle, followed by a slow spin (500-600 rpm). In addition, two synthetic bags containing approximately 5.0 g DM of each concentrate were washed through

the same cold rinse cycle to provide a measure of the immediately soluble fraction. After washing, the bags, string and sample were dried at 80°C to a constant weight prior to the removal of the string and the re-weighing of the bag and sample. The residue for each diet from each sheep at each time point was bulked and stored in an airtight container prior to analysis.

2.2.6.3 Analysis of the residues

Residues were analysed for N and residual DM as described in sections 2.3.4 and 2.3.2 respectively.

2.2.6.4 Calculations

The rate and extent of N degradation was determined by fitting data to the first order model of Ørskov and McDonald (1979).

$$P = a+b(1-e^{-ct})$$

Where P is the amount of food degraded at time t (h); a is the immediately soluble fraction; b is the insoluble but potentially degradable fraction and c is the constant rate of degradation of b.

The effective degradability (P) was calculated according to Ørskov and McDonald (1979) as:

$$P = a + \frac{(b \times c)}{(c + r)}$$

Where r is the rumen outflow rate per hour

2.3 SAMPLE ANALYSIS

2.3.1 Feed samples

Feed samples were taken as described in the respective experimental chapters. All feed samples were analysed for content of DM, ash, CP, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent insoluble nitrogen (ADIN).

2.3.2 Dry matter

Dry matter content was carried out according to the method of MAFF (1986). Sub-samples of material were analysed for DM content (g/kg) by accurately weighing approximately 1 g of sample into porcelain crucibles and then drying in an oven at 80°C to constant weight. The content of dry matter (g/kg) was calculated as:

$$\left\{ \frac{\text{weight of dry sample (g)}}{\text{weight of original sample (g)}} \right\} \times 1000$$

2.3.3 Ash

Ash content was carried out according to the method of MAFF (1986). Approximately 1 g of dried material was accurately weighed into a porcelain crucible and then heated to 450°C in a muffle furnace (Gallenkamp, size 3) for 16 hours. The ash remaining was then cooled in a desiccator to room temperature and reweighed. The content of ash (g/kg DM) was calculated as:

$$\left\{ \frac{\text{weight of ash (g)}}{\text{weight of orginal sample (g)}} \right\} \times 1000$$

2.3.4 Crude protein

Samples of approximately 0.5 - 1.0 g of material were analysed by an automated Kjeldahl procedure using a Tecator 1035 autoanalyser (Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, OX11 7NN). Samples were accurately weighed into digestion tubes containing two kjeltab catalyst tablets and then boiled in H_2SO_4 (6N, 14 ml) at 400°C for 45 mins. Samples were allowed to cool and then deionised water was added (75 ml). Crude protein content (total N x 6.25) was then estimated via back titration using HCl (0.2 M) as the titrant.

2.3.5 Ether extract

Ether extract was determined according to the method of MAFF (1986). Approximately 2-3 g of ground (1 mm screen) fresh material was accurately weighed into cellulose extraction thimbles which were then plugged with de-fatted cotton wool. Total fat was extracted by boiling samples in 25 ml of petroleum ether for 30 mins using a extraction unit (Tecator Soxtec 1043, Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, OX11 7NN). Samples were then rinsed for 15 mins. Final traces of the petroleum ether was allowed to evaporate in a fume cupboard. The fat content was calculated as:

$$\text{Ether extract (g/kg DM)} = \left\{ \frac{\text{Weight of fat (g)}}{\text{Weight of sample (gDM)}} \right\} \times 1000$$

2.3.6 Neutral detergent fibre (NDF)

Neutral detergent fibre concentration in feedstuffs was determined by the method of Van Soest *et al.* (1991). A sample (0.5 g) of ground, dried material was accurately weighed into a previously weighed crucible. The crucible was then secured into place in the fibretec apparatus (Tecator 1020, Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, OX11 7NN) and 25 ml of cold neutral detergent fibre solution added. Neutral detergent fibre solution was made by dissolving 93 g of disodium ethylene diamine tetra-acetate dihydrate (EDTA) and 34 g of

sodium borate in distilled water using gentle heating. To this, 150 g of sodium lauryl sulphate and 50 ml of 2-ethoxy ethanol were added. In a separate flask 22.8 g of anhydrous disodium hydrogen phosphate was dissolved in distilled water. The two solutions were then mixed and diluted to 5 litres. The pH was adjusted to lie between 6.9 and 7.1 and 25 ml was dispensed into each sample and 0.5 ml of anti-foaming agent (octanol).

The samples were boiled for 30 mins after which the heat was turned off and the samples washed (filtered) three times using 20 ml of hot (80°C) distilled water. Filtrates were discarded. Warm (55°C) deionised water was then added to the residues and 2 ml of α-amylase solution (2.2 g of α-amylase E.C.3.2.1.1 from *Bacillus sbtilis*) was dissolved in 99 ml of distilled water. This was then filtered and 11 ml of 2-ethoxy ethanol was added to the filtrate. Samples were then mixed and allowed to stand for 30 mins and then washed 3 times with hot (80°C) deionised water and once with 100% acetone (20 ml). Crucibles, containing residues were then removed from the fibretec apparatus, and dried at 100°C overnight in an oven. Crucibles were then cooled in a desiccator and reweighed. Samples were then heated to 550°C for 4 h, cooled in a desiccator and reweighed.

NDF content (g/kg DM) was calculated as:

$$\left\{ \frac{\text{residue weight (g) - ash content (g)}}{\text{Sample weight (g DM)}} \right\} \times 1000$$

2.3.7 Acid detergent fibre (ADF)

A sample (1 g) of ground dried material were accurately weighed into a previously weighed crucible. The crucible was then secured into place in the Fibretec apparatus (Tecator 1020, Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, OX11 7NN). To each sample was added 100 ml of acid detergent reagent [20 g of cetyle trimethylammonium bromide (CTAB)

dissolved in sulphuric acid (1 M)] and then boiled for 60 min, filtered and washed 3 times using hot (80°C) water (20 ml) and once with acetone (20 ml). Crucibles, containing residues were then removed from the fibretec apparatus, and dried at 100°C overnight in an oven. Crucibles were then cooled in a desiccator and reweighed. Samples were then heated to 550°C in a muffle furnace for 4 h, cooled in a desiccator and reweighed.

ADF content (g/kgDM) was calculated as:

$$\left\{\frac{\text{residue weight (g) - ash content (g)}}{\text{Sample weight (gDM)}}\right\} \quad \text{x } 1000$$

2.3.8 Acid detergent insoluble nitrogen (ADIN)

Kjeldahl analysis was carried out using a Tecator auto analyser as described in section 2.3.4 on the residue obtained after an ADF procedure prior to heating to 550°C (section 2.3.7).

2.3.9 Immunoglobulin G content of ewes colostrum

The IgG concentration of colostrum samples was determined by the method of Fahey and McKelvey (1965). The method involved antigen diffusing radially from a cylindrical well through an agarose gel containing monospecific antibody. After complete diffusion, antigen-antibody complexes formed a precipitin ring.

Thawed samples of colostrum that had been taken immediately after parturition were diluted 1 in 60 with distilled water. Equal amounts (200 µl) of the diluted colostrum and of bovine serum albumin (BSA; The Binding Site, Birmingham, England) solution were mixed in a micro-centrifuge tube. The radial immunodiffusion plate (RID; The Binding Site, Birmingham, England) was removed from the foil container and the lid removed to allow any water droplets to evaporate and 5 µl of the diluted colostrum from the micro-centrifuge tube was placed into

each well. The ring diameter was measured after 72 hours and again after 96 hours to check that the samples had fully diffused. After complete diffusion a linear relationship existed between the square of the ring diameter (ring diameter^2) and the antigen concentration. By using three calibrators of known IgG concentration (100 mg/l, 600 mg/l and 1000 mg/l), a graph of the linear relationship between the square of the ring diameter and the known antigen concentration in each calibrator was constructed. The IgG concentration in the unknown, diluted samples was then determined from the graph, using the ring diameter measured. Multiplying by the dilution factors (1 in 60 with distilled water and then 1 in 2 with bovine serum albumin (BSA) solution; therefore, overall dilution was 1 in 120) gave the concentration of IgG in the colostrum.

For colostrum samples taken at 16 hours *post partum* the same procedure as above was carried out, but the samples were diluted 1 in 20 with distilled water and 1 in 2 with BSA solution, giving an overall dilution of 1 in 40.

2.3.10 Lactose content of ewes colostrum.

The following method was adapted from that of Teles *et al.* (1978). Approximately 2 g of colostrum were accurately weighed into a 50 ml volumetric flask and diluted to 50 ml with distilled water. After thorough mixing, a 2.5 ml aliquot of the diluted sample was transferred to a 15 ml centrifuge tube and then 0.2 ml of 5 % zinc sulphate followed by 0.2 ml of 4.5 % barium hydroxide was added. The sample was mixed well and allowed to stand for 2 minutes followed by centrifuging (Beckman Avanti 30 centrifuge) at 2800 rpm (1402 x g) for 40 seconds. Following centrifuging, 1 ml of the supernatant was transferred to a stoppered test tube and 2.5 ml of Teles reagent added. Teles reagent has a two day shelf life and must be stored away from light. It was prepared by mixing 1 volume of 1 % phenol solution, 2 volumes

of 5 % sodium hydroxide, 2 volumes of 1 % picric acid and 1 volume of 1 % sodium hydrogen sulphate in order. The tube containing the sample supernatant and the Teles reagent was then immersed in a boiling water bath for exactly 6 minutes followed by immediate cooling in cold water. The resulting solution was quantitatively transferred to a 25 ml volumetric flask and diluted to volume with distilled water. The absorbance of the resulting solution was read at 520 nm (Beckman, DU 640) against a similarly treated reagent blank, in which 2.5 ml of distilled water substituted for the sample. The results were compared with a standard solution of lactose (1 mg/ml) that had undergone the same procedure.

Lactose concentration was calculated as:

$$\text{Lactose concentration (g/l)} = \frac{\text{absorbence of sample @ 520nm}}{\text{absorbence of standard @ 520nm}} \times \frac{50}{\text{sample wt (g).}}$$

2.3.11 Fat content of ewes colostrum

Fat content of ewes colostrum was determined according to the method of MAFF (1986). Colostrum samples were thawed in a 20°C water bath and, after mixing, were diluted 1 in 3 with distilled water. Sulphuric acid (10 ml) was measured into the Gerber butyrometer tube and then 10.94 ml of diluted colostrum was added using a milk pipette. Care was taken to hold the pipette at an angle of 30°C to the vertical allowing the diluted colostrum to enter without mixing with the sulphuric acid. Subsequently, 1 ml of amyl alcohol was measured into the tube, leaving three distinct layers in the tube. At all times care was taken not to wet the neck of the tube with either the sulphuric acid, colostrum or the amyl alcohol. A lock stopper was inserted until the rim was in contact with the neck of the butyrometer and after placing the Gerber tube in a protected stand it was shaken. When all white particles had disappeared from the solution, the butyrometer was immediately placed in a centrifuge for 4-5 minutes at 1100 rpm (311 x g). The butyrometer was then removed and placed into a water bath for 3-10 minutes at 65±2°C,

which contained sufficient water to allow the top of the fat column to be immersed. After removal from the water bath, the fat content was then read of the graduated scale and having noted the reading, the butyrometer was placed back in the water bath and the procedure repeated. The differences between the readings were between ± 0.05%. Multiplying the mean of the two readings by 30 gave the fat content of the colostrum sample in g/kg.

2.3.12 Milk analysis

Ewe milk samples were analysed for fat, CP, lactose and solids not fat (SNF; g/kg) using a Dairylab 2 infrared milk analyser. The Dairylab 2 was calibrated using standards containing known concentrations of fat ranging from 30 to 260 g fat / kg. The standards were supplied by Quality Management, Trenslo House, Tile Street, Bury, Lancashire, UK. Samples were allowed to defrost at room temperature and then heated to 40°C in a circulating water bath. Samples were homogenized for 30 seconds and then a 1 ml sub-sample was injected into the milk analyser. Both the homogenizer and the sampling probe were washed with warm (40°C) deionised water between samples.

2.3.13 Blood analysis

Blood samples were collected into 7 ml evacuated tubes (Vacutainer) containing the anticoagulants lithium heparin and potassium oxalate at the times described in the respective experimental chapters. Samples were centrifuged at 3000 rpm for 15 minutes (Beckman Avanti 30 centrifuge) and the plasma was transferred to micro-centrifuge tubes for storage at -80°C. All blood plasma samples were thawed slowly to room temperature. Samples collected into vacutainers tubes that contained lithium heparin as the anti-coagulent were analysed for total protein (g/l), albumin (g/l) and urea-N (mmol/l). Samples collected into vacutainers tubes that contained potassium oxalate as the anti-coagulent were analysed for BHB (mmol/l), NEFA

(mmol/l) and glucose (mmol/l). Total protein, albumin, urea nitrogen and glucose analysis was carried using Bayer methods and reagents (Bayer, 511 Benadict Avenue, Tarrytown, New York, USA. NEFA and BHB analysis was carried out using Wako methods and reagents (Wako Chemicals, Nissanstr. 2, D-41468 Neuss, Germany) and Sigma (Sigma Diagnostics. PO Box 14508, St. Louis. MO63178, USA) reagents respectively. All analysis was carried out on a Bayer Technicon RA-1000 autoanalyzer (Bayer plc, Strawberry Hill, Newbury, Berkshire, GR14 1LA).

CHAPTER 3

THE EFFECTS OF SOURCE OF ENERGY AND PROTEIN ON THE METABOLISM AND PERFORMANCE OF HOUSED, STRAW FED, PREGNANT AND LACTATING EWES

3.1 INTRODUCTION

During late pregnancy and early lactation the protein requirements of the ewe may often exceed the amount supplied by microbial protein from the rumen (Robinson, 1983b). It has therefore become common practice, after maximising microbial crude protein production, to supplement the diet of the ewe in late pregnancy and early lactation with UDP. Fishmeal is a suitable protein source in this situation, providing high levels of UDP with a high relative value (Sheehan and Hanrahan, 1989).

More recently, concerns have been expressed over the effects of industrial fishing (House of Lords, 1996) and of the effects of animal proteins in ruminant diets (GAFTA, 1997). However, the use of vegetable protein sources (eg soya-bean meal), when used as alternatives to fishmeal have the disadvantage of being relatively high in rumen degradable and consequently low in undegradable protein. This has resulted in methods of protection from rumen degradation being developed (Barry, 1976; Antoniewicz *et al.*, 1992; Hadjipanayiotou, 1992; Subuh *et al.*, 1994; Hadjipanayiotou and Photiou, 1995; Rodehutscord *et al.*, 1999). Additionally, vegetable protein sources, in particular soya-bean meal, are lacking in their content of certain essential amino acids, particularly methionine (Storm and Ørskov, 1984). The supply of essential amino acids and the relative value of the protein can be improved by the addition of rumen protected amino acids (Undi *et al*, 1996).

It has also been shown that concentrates can be partially replaced by supplementary feedblocks, based on soluble sugars and urea, with no adverse effects on production but simplifying the feeding regime (Chapple *et al.*, 1996). Feedblocks have been evaluated in hill and upland situations (Ducker *et al.*, 1981; Lawrence and Wood-Gush, 1988), but their suitability as a supplement for lowland ewes in late pregnancy has not been studied.

The objectives of the current experiment were to compare the effects of feeding barley based concentrates containing either fishmeal or formaldehyde protected soya-bean meal with added amino acids as the main protein source and to compare the partial replacement of concentrates with a supplementary feed based on soluble sugars and urea on the performance and metabolism of pregnant and lactating ewes.

3.2 MATERIAL AND METHODS

3.2.1 Animals

At 103 days of gestation, 72 twin-bearing Charollais x Lleyn (n=16), Charollais x Cambridge (n=16) and Friesland x Lleyn (n=40) ewes were randomly blocked and allocated to one of four dietary treatments by breed, age (considered as either shearlings or older) weight and condition score. Ewes were in lamb to Charollais and / or Suffolk tups.

3.2.2 Diets

The dietary treatments were concentrate containing fishmeal (50 g/kg; F) or a concentrate replacer containing formaldehyde treated soya-bean meal (70 g/kg; S) with added amino acids (0.75 g/kg) and Diamond V 'XP' Yeast (2.5 g/kg; non-viable *Saccharromyces cerevisiae;* Rumenco, Burton on Trent, England; Table 3.1). These were fed either alone or at a reduced rate (0.75 of diets F or S) with *ad libitum* Rumevite Standard Feedblocks (262 gCP/kg DM;

9.0 MJ ME/kg DM; Rumenco, Burton on Trent, England; Table 3.1 (B); in a 2 (protein source) by 2 (with or without feedblocks) factorial design. Formaldehyde treatment was carried out by applying 9.3 litres of a 30 % formaldehyde solution to one tonne of soya-bean meal, which is equivalent to 2.8 g formaldehyde per kg of soya-bean meal (5.4 g/kg CP). Concentrates were formulated to be isoenergetic (12.6 MJ ME/kg DM) and to produce an ERDP:FME ratio greater than 10.5 g/MJ in the total diet. All concentrates were supplied on the same incremental scale (Table 3.2). The experiment ran from seven weeks prior to six weeks post lambing.

Table 3.1 *Dietary composition (g/kg) and predicted chemical composition (g/kg DM) of concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) and the dietary composition (g/kg) and predicted chemical composition (g/kgDM) of the feedblocks offered to ewes during late pregnancy and early lactation*

	Treatment				
	F	S	FB	SB	Feedblock
Ingredient (g/kg)					
Rolled barley	495	455	430	380	421
Rapeseed meal	115	135	70	100	53
Soya-bean meal	115	115	225	225	
Fishmeal	50		50		
Formaldehyde treated soya-bean meal		70		70	
Sugar beet pulp shreds	100	100	100	100	
Molasses	100	100	100	100	184
Minerals / vitamins	25	25	25	25	52
Urea					58
Rock salt					53
Diamond XP yeast*					53
Calmag*					21
Lignobond DD*					105
ME (MJ/kg DM)	12.6	12.5	12.7	12.6	9.0
CP	233	231	267	266	262
EE	18	16	18	15	10
NDF	170	207	153	194	201
FME (MJ/kg DM)	12.2	12.1	12.3	12.1	8.4
ERDP[1]	154	151	169	168	209
ERDP[2]	139	135	149	148	205
DUP[1]	52	57	65	70	9
DUP[2]	65	71	82	88	12

[1] calculated at a rumen outflow rate (r) = 0.05

[2] calculated at a rumen outflow rate (r) = 0.08

*= Rumenco, Burton on Trent, England

Table 3.2 *Amount of concentrate (kg fresh weight/ewe/day) containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) and fed to ewes during late pregnancy and early lactation*

	Diet			
Weeks *pre/post partum*	F	S	FB	SB
-7 to -6	0.6	0.6	0.45	0.45
-6 to -4	0.8	0.8	0.6	0.6
-4 to -2	1.0	1.0	0.75	0.75
-2 to 0	1.2	1.2	0.9	0.9
0 to +5	1.8	1.8	1.35	1.35
+5 to +6	1.2	1.2	0.9	0.9

3.2.3 Procedure and measurements

At 7 weeks *pre partum*, eight ewes per treatment were individually penned and bedded on sawdust, with the remaining 10 allocated to one of four group pens. All ewes were offered straw daily, as a single feed at 0800 hours at proportionally 1.25 of the previous calculated intake. The straw refusals were removed and weighed (±10 g) at 0730 hours on Mondays, Wednesdays and Fridays. Straw offered was sampled weekly (Wednesday) by taking 6 equal samples from six separate bales and a 200 g sample of refused straw was taken from individually penned sheep on Mondays. Concentrates were fed in three equal meals (0830, 1300 and 1630 hours) from lambing to 5 weeks *post partum* and in two equal meals (0830 and 1630 hours) at all other times. Concentrates were sampled weekly (Wednesday) by taking equal amounts (200 g) from four separate 25 kg bags. Feedblocks were offered *ad libitum* to the appropriate ewes using a flexibag feeder (Rumenco, Burton on Trent, England) hung on the side of the pen. Feedblocks were weighed (±10 g) weekly, each Wednesday, at 1400 hours. This was prior to obtaining four equal samples from four separate feedblocks. All samples of straw, concentrate and feedblock were stored at 4°C in airtight containers until subsequent analysis.

Ewe liveweight and body condition score were measured at weekly intervals, lamb birth weight and weekly weight and colostrum production were measured by the methods given in Chapter 2. Blood samples were taken at 10 am during weeks 6, 4, 2 and 1 *pre partum* and at weeks 1, 2, 4 and 6 of lactation by the methods described in Chapter 2.

Samples of colostrum were analysed for DM, fat, CP, IgG, lactose and ash (Chapter 2). Blood plasma samples were analysed for total protein, albumin, urea nitrogen, BHB, NEFA and glucose as described in Chapter 2.

3.2.4 The *in-situ* rumen degradability of nitrogen

The *in-situ* nitrogen degradability in the four concentrate feeds was measured using four rumen cannulated sheep.

3.2.4.1 Experimental animals, treatment and design

Four wether sheep aged 6 years with an average weight of 81 kg (s.d. 2.9 kg) and fitted with permanent rumen cannulae of 39 mm internal diameter, were assigned to a 4 x 4 latin square design and housed in individual, slatted floor pens, with free access to water and mineral licks. Animals were introduced to their surroundings and diet two weeks prior to the insertion of polysynthetic fibre bags.

A basal concentrate diet was formulated to be a mean of the diets (concentrate and feedblock) used in the production trial (Table 3.3).

Table 3.3 *Dietary composition (g/kg) of the basal concentrate fed to the rumen-cannulated wethers*

	Composition (g/kg)
Ingredient	
Barley	471
Molasses	109
Sugar beet pellets (molassed)	90
Fishmeal	24
Formaldehyde treated soya-bean meal	24
Rapeseed meal	111
Soya-bean meal	158
Urea	13

The wethers were then offered barley straw to achieve a diet with a ratio of barley straw:concentrate of 0.43:1, which was the same as the mean ratio consumed by the ewes in the production experiment. Concentrates were offered as a coarse mix in two equal feeds at 0830 and 1630 hours and straw at 0835 and 1635 hours. Diets were fed at 1.1 x maintenance.

Samples were collected and processed as described in Chapter 2.

3.2.5 Statistical analysis

The experiment was designed as a 2 x 2 factorial design with main effects of Protein source (F *v.* S) and Feedblock (with or without feedblock) and interaction (Int) between protein source and feedblock. Treatment differences in litter birth weight and lamb growth rate were analysed using number of males and the number of Suffolk cross lambs in the litter as co-variates. Lamb growth rate was estimated by linear regression. All statistical analyses was performed by analysis of variance (ANOVA) using Genstat 5 release 3.2 (Lawes Agricultural Trust, 1995).

3.3 RESULTS

The data from two ewes were excluded from the results. Both ewes (fed diets FB and SB) developed mastitis during the last week of pregnancy. All data collected during lactation from three other ewes (except colostrum data) were excluded from the results (fed diets F, S and SB) because they all only reared one live lamb to 6 weeks of age.

3.3.1 Diet composition

The analysed chemical composition of concentrates, feedblocks and straw are presented in Table 3.4. The chemical composition of all concentrates was similar, with mean values for DM of 909 g/kg, EE 39 g/kg DM, ash 74 g/kg DM, NDF 167 g/kg DM, ADF 87 g/kg DM and ADIN 1.68 g/kg DM. The higher concentrations of CP in FB and SB compared to F and S were similar to that predicted (Table 3.1). The chemical composition of the fresh straw and the refusals were both very similar. The chemical composition of the feedblocks was also close to the predicted values.

Table 3.4 *Actual chemical composition (g/kg DM or MJ/kg DM) of concentrates containing fishmeal (F), protected soya-bean meal (S), formulated for feeding alone or with feedblocks (B) and actual chemical composition (g/kg DM or MJ/kg DM) of feedblocks along with fed and refused winter barley straw*

	F	S	FB	SB	Feedblocks	Straw	Refused straw
Chemical composition							
g/kg DM (or MJ/kg DM)							
DM (g/kg)	920	918	876	921	897	872	850
CP	237	231	270	266	241	36	32
EE	20	18	21	16	7	12	10
Ash	82	65	79	69	240	47	50
NDF	170	162	165	172	105	784	750
ADF	84	91	81	93	42	486	492
ADIN	1.69	1.41	1.91	1.69	1.20	1.3	1.4

3.3.2 Nitrogen degradability of the concentrates

N degradability coefficients of the four concentrates are presented in Table 3.5. Concentrates containing treated soya-bean meal (S and SB) had a similar soluble N fraction (*a*) and a similar rate of N degradation (*c*) of the potentially degradable N fraction (*b*) as concentrates containing fishmeal (F and FB). The effective N degradability (P) of each concentrate was reduced at the higher outflow rate with mean values of 0.65 and 0.57 at outflow rates of 0.05 and 0.08 respectively. The effective N degradability (P) at a given outflow rate was similar for all the four concentrates.

Table 3.5 *Nitrogen (N) degradability coefficients for concentrates containing fishmeal (F) or protected soya-bean meal (S) formulated for feeding alone or with feedblocks (B) to ewes in late pregnancy and early lactation*

	F	S	FB	SB	s.e.d.
a	0.24	0.23	0.21	0.18	0.011
b	0.67	0.72	0.72	0.80	0.028
c	0.073	0.073	0.087	0.070	0.0121
a+b	0.91	0.95	0.93	0.98	0.026
r^2	95.8	96.3	97.1	96.7	
Effective N degradability (P):-					
r=0.05	0.64	0.65	0.67	0.65	0.025
r=0.08	0.56	0.58	0.57	0.55	0.027

Where *a* is the immediately soluble fraction, *b* is the insoluble but potentially degradable fraction, *c* is the constant rate of degradation of *b* and r is the rumen outflow rate per hour. Effective N degradability (P) was calculated according to the equations given in Chapter 2.

3.3.3 Feed and nutrient intake

Mean intakes of *pre partum* DM, ME, DUP and MP were 1.37 kg/d, 13.7 MJ/d, 49 g/d and 127 g/d respectively. Inclusion of fishmeal (diets F and FB) in the concentrate resulted in ewes having a significantly higher *pre partum* intake of DM (1.45 *v.* 1.29 kg/d), ME (14.3 *v.* 13.1 MJ/d), DUP (52 *v.* 46 g/d) and MP (134 *v.* 120 g/d; Table 3.6) compared to ewes fed diets containing the soya-bean based diet (S and SB). This was mainly due to a significantly higher intake of feedblocks by ewes fed diet FB (0.59 kg DM/d) compared to those fed diet SB (0.35

kg DM/d; $P<0.001$) and a higher mean intake of straw in ewes fed diets containing fishmeal (F and FB) compared to those fed the soya-bean based diet (S and SB; 0.44 *v.* 0.40 kg DM/d respectively). Partial replacement of concentrates with feedblocks resulted in an increase in the intake of DM (1.22 *v.* 1.52 kg/d; $P<0.001$) and ME (12.9 *v.* 14.5; $P<0.01$) in the *pre partum* period, but did not significantly affect DUP or MP intake.

Mean *post partum* intake of dry matter was higher than the *pre partum* intake in ewes fed any of the four diets (Table 3.6). Ewes fed concentrates containing fishmeal (F and FB) tended to have an increased DM intake (2.34 *v.* 2.10 kg/d; $P=0.093$) and had a significantly increased intake of ME (23.9 *v.* 22.1 MJ/d; $P<0.05$), DUP (126 *v.* 118 g/d; $P<0.05$) and MP (270 *v.* 251 g/d; $P<0.01$), compared to those fed diets containing a soya-bean meal diet (S and SB). Increases in nutrient supply due to the inclusion of fishmeal was due to increases in mean intake of straw (0.72 *v.* 0.63 kg DM/d) for diets F and FB *v.* S and SB respectively along with a significantly higher intake of feedblock (0.66 *v.* 0.37 kg DM/d; $P<0.01$) for diets FB and SB respectively. Partial replacement of concentrates with feedblocks significantly increased the *post partum* intake of DM (2.36 *v.* 2.07 g/d; $P<0.001$) and MP (270 *v.* 241 g/d; $P<0.05$), but had no effect on the intake of DUP or ME.

Table 3.6 Pre partum *and* post partum *intake of concentrate (kg/d), straw (kg/d), feedblock (kg/d), total dry matter (DM; kg/d), metabolisable energy (ME; MJ/d) and metabolisable protein (MP; g/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Pre partum:-								
Concentrate	0.82	0.82	0.62	0.62	-	-	-	-
Straw	0.43	0.37	0.44	0.42	0.064	NS	NS	NS
Feedblock	-	-	0.59	0.35	0.018	***	-	-
DM	1.25	1.19	1.65	1.39	0.075	*	***	NS
ME	13.0	12.7	15.5	13.4	0.61	**	**	NS
DUP	53	45	51	47	2.2	***	NS	NS
MP	129	118	138	122	5.9	**	NS	NS
Post partum:-								
Concentrate	1.47	1.47	1.10	1.10	-	-	-	-
Straw	0.64	0.56	0.80	0.69	0.175	NS	NS	NS
Feedblock	-	-	0.66	0.37	0.102	**	-	-
DM	2.11	2.03	2.56	2.16	0.171	NS	***	NS
ME	22.7	22.0	25.0	22.1	1.19	*	NS	NS
DUP	131	118	121	118	4.5	*	NS	NS
MP	256	246	283	256	9.2	**	*	NS

3.3.3.1 Intake of concentrate, feedblock, straw, dry matter, metabolisable energy, digestible undegradable protein and metabolisable protein

Straw intake increased in ewes on all dietary treatments from 7 (overall mean of 0.17 kg DM/d) to 6 (0.46 kg DM/d) weeks *pre partum* and declined slightly towards parturition (0.44 kg DM/d at 1 week *pre partum*; Figure 3.1). In lactation, straw intake increased in ewes fed all diets as lactation progressed from the first (0.44 kg DM/d) to the fourth week of lactation (0.73 kg DM/d). Subsequently, intake of straw declined slightly in ewes fed diet FB (from 0.93 kg DM/d to 0.88 kg DM/d from the fourth to the sixth week of lactation), but continued to increase in ewes fed all other treatments, reaching 0.81, 0.64 and 0.86 kg DM/d during the sixth week of lactation for ewes fed diets F, S and SB respectively. There was no significant effect of either the protein type or feedblocks on the daily straw intake by ewes in the *pre partum* or *post partum* period.

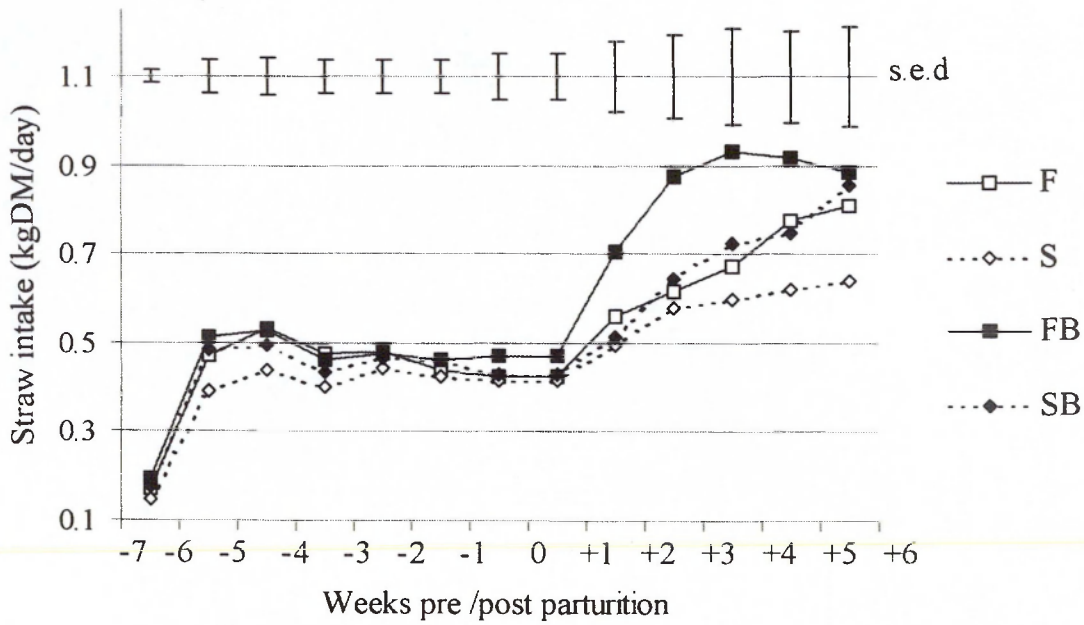

Figure 3.1 Intake of straw (kg DM/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

Feedblock intake increased in ewes fed diet FB and SB from seven weeks *pre partum* (mean intake of 0.45 kg DM/d) to 6 weeks *pre partum* (0.56 kg DM/d), and subsequently declined towards parturition (0.31 kg DM/d). Intake increased as lactation progressed and reached a maximum in the sixth week of lactation (0.77 kg DM/d; Figure 3.2). No significant effect of diet on block intake was observed at 7 weeks *pre partum*. However, significantly higher intakes of feedblock were observed in ewes fed a diet containing fishmeal compared to those fed a diet containing a soya-bean meal diet from week 6 to week 1 *pre partum* ($P<0.05$). In lactation, ewes fed concentrate containing fishmeal (FB) continued to have a significantly higher feedblock intake than those fed diets containing a soya-bean based diet (SB) from the first to the sixth week of lactation ($P<0.05$).

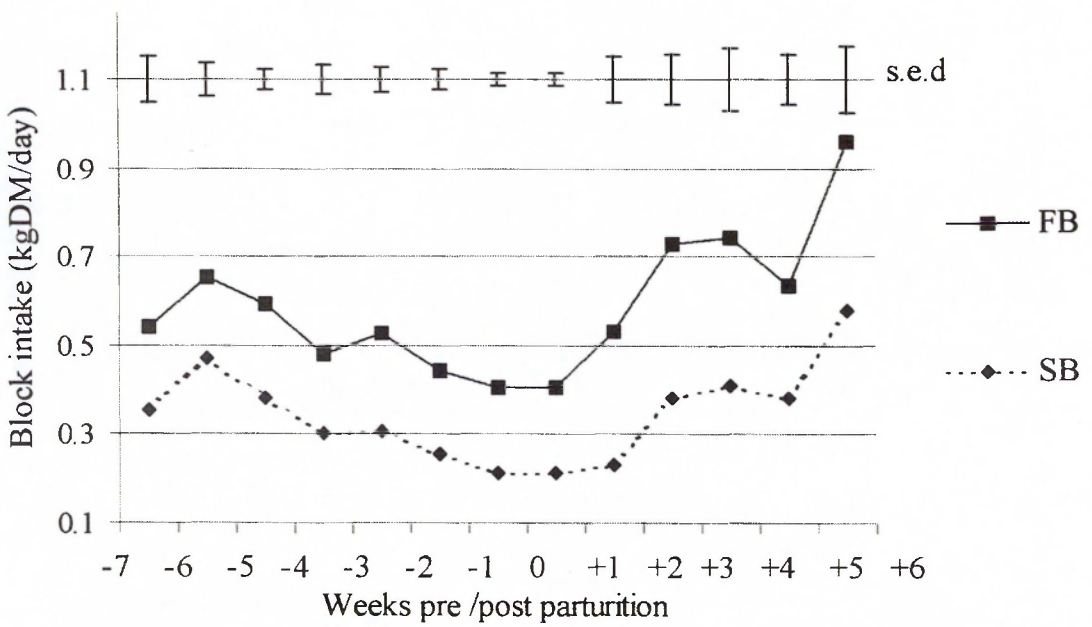

Figure 3.2 Intake of feedblock (kgDM/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The total DM intake increased in ewes on all treatments from 7 (overall mean of 0.85 kg DM/d) to 6 (1.32 kg DM/d) weeks *pre partum* and increased slightly towards parturition (1.50 kg DM/d at 1 week *pre partum*; Figure 3.3). In lactation, total DM intake increased in ewes fed all diets as lactation progressed from the first (1.96 kg DM/d) to the fourth week of lactation (2.38 kg DM/d). Subsequently, intake of total DM declined in ewes not offered feedblocks (diets F and S) to 1.76 kg DM/d in the sixth week of lactation, whilst total DM intake in the sixth week of lactation remained high in ewes offered feedblocks (mean intake of 2.45 kg DM/d for ewes fed diets FB and SB). Higher intakes of total DM were observed in ewes fed diets containing fishmeal (diets F and FB) compared to those fed diets containing a soya-bean meal diet (diets S and SB) at 5, 4 and 3 weeks *pre partum* and during the second and third ($P<0.05$) weeks of lactation. Higher intakes of DM were also observed in ewes offered feedblocks (diets FB and SB) compared to those offered no feedblocks (diets F and S) from 7 to 2 weeks *pre partum* and during the third, fourth and sixth week of lactation ($P<0.05$).

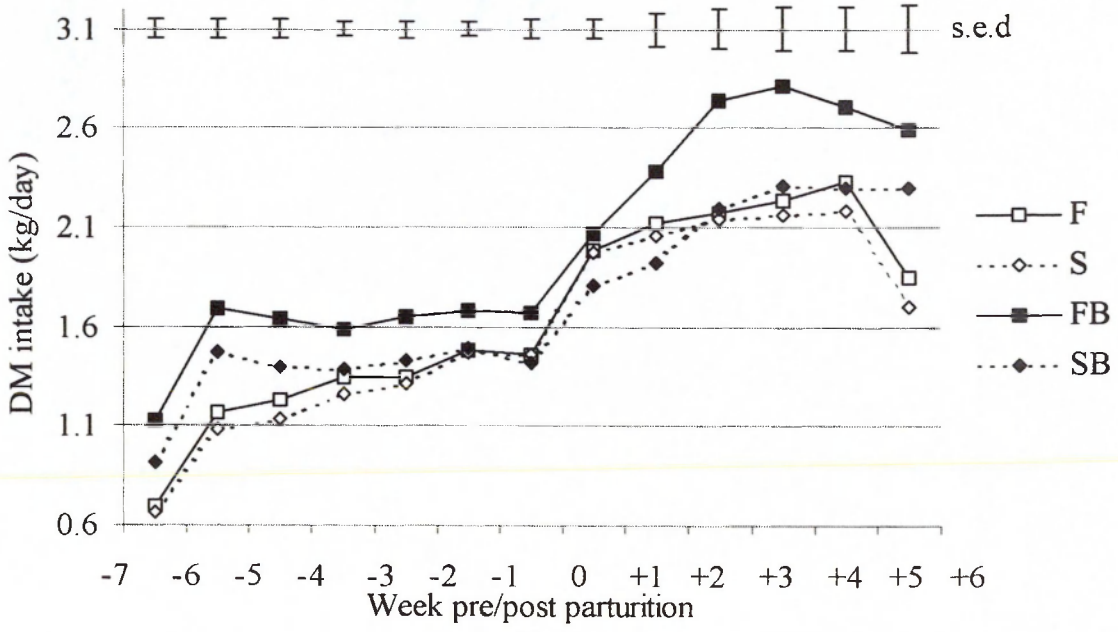

Figure 3.3 Intake of total dry matter (DM; kg/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The total ME intake increased in ewes on all treatments from week 7 (overall mean of 8.84 MJ/d) to parturition (15.7 MJ/d at 1 week *pre partum*; Figure 3.4). In lactation, ME intake increased in ewes fed all diets as lactation progressed from the first (21.4 MJ/d) to the fifth week of lactation (24.3 MJ/d). Subsequently, intake of ME declined in ewes fed all treatments to 20.2 MJ/d in the sixth week of lactation. Higher ME intakes were observed in ewes fed diets containing fishmeal (diets F and FB) compared to those fed diets containing a soya-bean meal (diets S and SB) from week 5 to week 1 ($P<0.05$) *pre partum* and form the first to the fifth week of lactation ($P<0.05$). Higher intakes of ME were also observed in ewes offered feedblocks (diets FB and SB) compared to those fed offered no feedblocks (diets F and S) from week 7 to week 3 *pre partum* and during the first and sixth week of lactation ($P<0.01$).

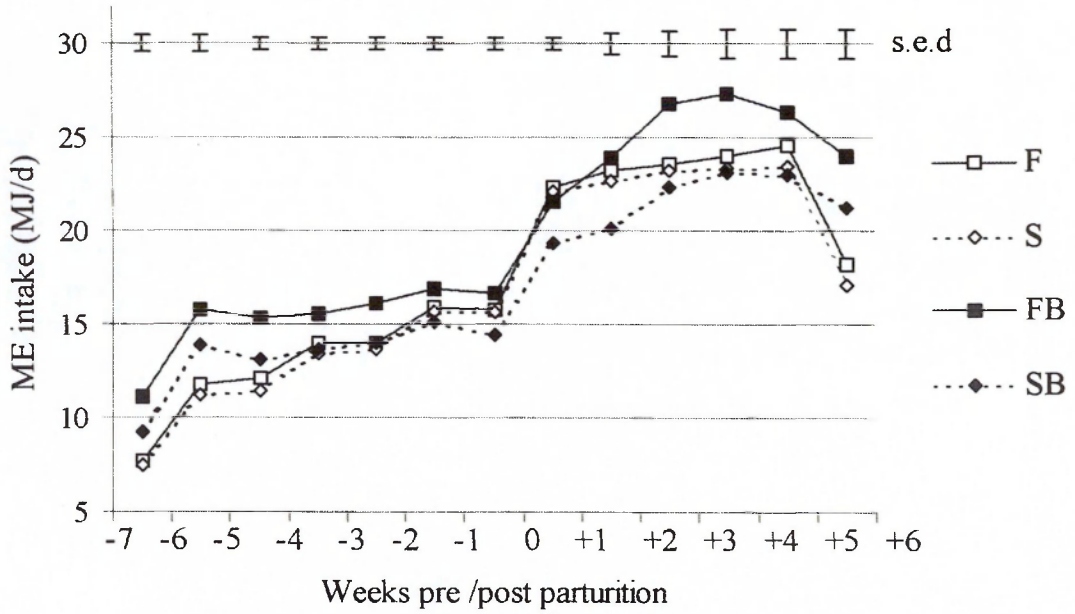

Figure 3.4 Intake of metabolisable energy (ME; MJ/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The total DUP intake increased in ewes on all treatments from a mean of 20 g/d at 7 weeks *pre partum* to 63 g/d at 1 week *pre partum*; Figure 3.5. In lactation, DUP intake increased steadily in ewes fed all diets as lactation progressed, with mean values of 118 g/d and 133 g/d in the first and the fifth week of lactation. Subsequently, intake of DUP declined sharply in ewes fed all treatments to a value of 91 g/d in the sixth week of lactation. Higher intakes of DUP were observed in ewes fed diets containing fishmeal (diets F and FB) compared to those fed diets containing a soya-bean meal (S and SB) from week 7 to week 1 *pre partum* ($P<0.05$) and from the first to the fifth week of lactation ($P<0.05$). Higher intakes of DUP were also observed in ewes offered feedblocks (diets FB and SB) compared to those not offered feedblocks (F and S) during weeks 7 and 6 *pre partum* and during the sixth week of lactation ($P<0.05$). However, lower intakes of DUP were seen in ewes offered feedblocks (FB and SB) compared to those offered no feedblocks (F and S) at week 2 ($P<0.01$) and week 1 *pre partum* ($P<0.01$) and during the first and second ($P<0.001$) week of lactation.

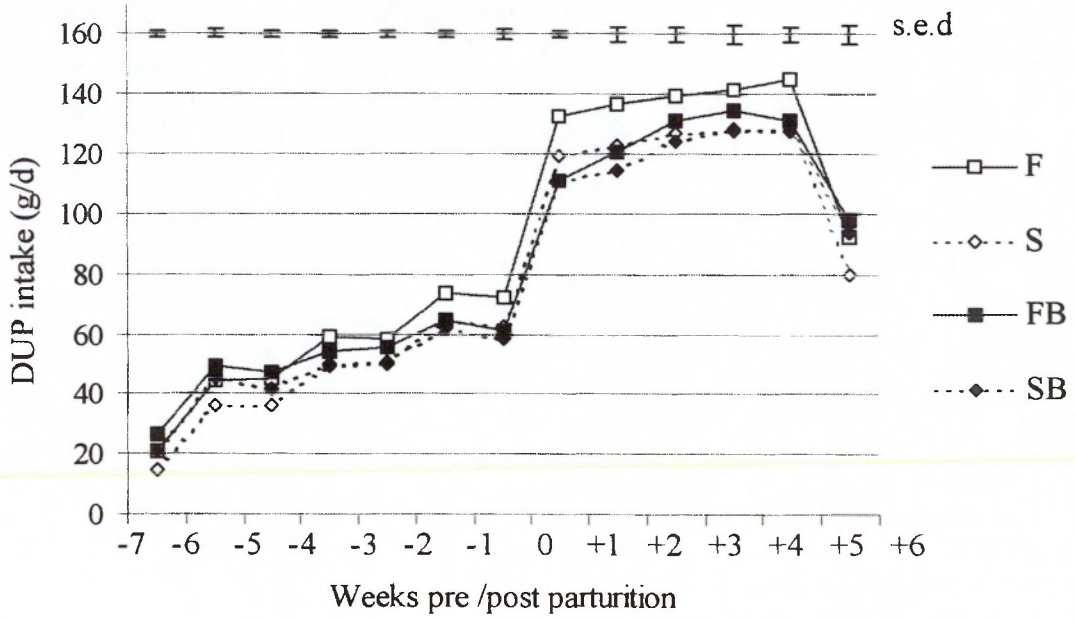

Figure 3.5 Intake of digestible undegradable protein (DUP; g/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The total MP intake increased in ewes on all treatments from 7 (overall mean of 67 g/d) to parturition (154 g/d at 1 week *pre partum*; Figure 3.6). In lactation, MP intake increased steadily in ewes fed all diets as lactation progressed from 250 g/d during the first to 278 g/d during the fifth week of lactation. Subsequently, intake of MP declined sharply in ewes fed all treatments to 211 g/d in the sixth week of lactation. Significantly higher intakes of MP were observed in ewes fed diets containing fishmeal (diets F and FB) compared to those fed diets containing a soya-bean meal (diets S and SB) from week 7 to week 1 *pre partum* ($P<0.05$) and from the first to the fifth ($P<0.05$) week of lactation. Higher intakes of MP were also observed in ewes offered feedblocks (diets FB and SB) compared to those offered no feedblocks (diets F and S) from 7 to 5 weeks *pre partum* and during the third, fourth, fifth and sixth week of lactation ($P<0.05$). However, lower intakes of MP were observed in ewes offered feedblocks (diets FB and SB) compared to those offered no feedblocks (diets F and S) at 1 week *pre partum* and during the first week of lactation ($P<0.05$).

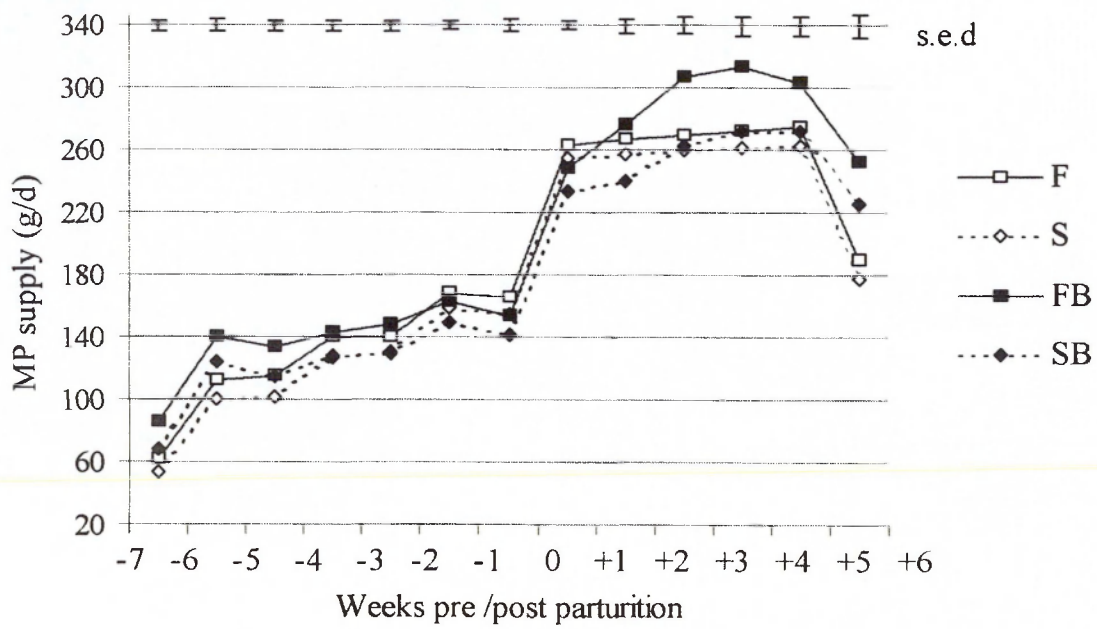

Figure 3.6 Intake of metabolisable protein (MP; g/d) in ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

3.3.4 Ewe weight and condition score

The initial, pre-treatment (7 weeks *pre partum*) weight and condition score, together with effect of treatment on the change in *pre partum* weight and condition score (7 to 1 weeks *pre partum*) are presented in Table 3.7. At the beginning of the experiment, ewes in all treatment groups had similar weights and condition scores (CS). Both protein source and the partial replacement of concentrates with feedblocks had no significant effect on the weight and CS of ewes at 1 week *pre partum* or on the *pre partum* weight and condition score change. Weight and condition score was gained *pre partum* and lost *post partum* in ewes fed all diets (Table 3.7). Whilst there was no effect of either protein source or feedblocks on weight immediately (at 12 hours) *post partum* or on *post partum* weight change, feeding concentrates containing fishmeal tended to increase the condition score immediately *post partum* (P=0.053) and increased the amount of condition lost between parturition and 6 weeks *post partum* (P<0.05).

Table 3.7 *Weight and condition score (CS) at 7 and 1 week* pre partum, *immediately post parturition and at 6 weeks post partum(kg), and* pre partum *(7 to 1 week* pre partum*) and* post partum *(lambing to 6 weeks post lambing) weight (kg/week) and CS (units/week) change of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S) formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Pre partum weight:-								
At 7 weeks *pre partum*	73.5	73.8	73.8	73.8	1.20	NS	NS	NS
At 1 week *pre partum*	82.8	82.0	83.3	81.9	1.50	NS	NS	NS
Pre partum change	+9.3	+8.2	+9.5	+8.5	1.03	NS	NS	NS
Pre partum CS:-								
At 7 weeks *pre partum*	2.50	2.42	2.50	2.47	0.088	NS	NS	NS
At 1 week *pre partum*	3.01	2.97	3.14	2.97	0.129	NS	NS	NS
Pre partum change	+0.58	+0.56	+0.64	+0.50	0.143	NS	NS	NS
Post partum weight:-								
Immediately *post partum*	71.6	70.5	70.6	69.4	1.52	NS	NS	NS
At 6 weeks *post partum*	64.0	63.1	62.8	62.2	1.85	NS	NS	NS
Post partum change	-7.6	-7.3	-8.1	-7.7	1.31	NS	NS	NS
Post partum CS:-								
Immediately *post partum*	2.78	2.56	2.64	2.56	0.109	NS	NS	NS
At 6 weeks *post partum*	2.47	2.41	2.32	2.47	0.094	NS	NS	NS
Post partum change	-0.31	-0.14	-0.32	-0.08	0.127	*	NS	NS

3.3.5 Colostrum production

3.3.5.1 Yield of Colostrum

At birth, the overall mean yield of colostrum was 533 g (s.d. 343.2; Table 3.8). Mean secretion rate at 12-16 hours *post partum* was 103 g/h (s.d. 49.1). There was no significant effect of treatment on any of the parameters measured.

Table 3.8 *Initial yield of colostrum (g), subsequent secretion rates (g/hour) and calculated 24 hour colostrum yield (g) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Initial yield	576	568	521	468	109.1	NS	NS	NS
Secretion rate	115	93	97	107	14.8	NS	NS	NS
24 h yield	3228	2784	2745	2922	375.3	NS	NS	NS

3.3.5.2 Colostrum composition and component yield at parturition

There was no significant effect of dietary treatment on the initial concentrations of DM, CP, fat, or lactose in colostrum (Table 3.9). Ewes fed diets containing fishmeal (F and FB) produced colostrum with a lower concentration ($P<0.05$) and yield ($P=0.076$) of ash compared to those ewes fed concentrate containing a soya-bean based diet (S and SB). The concentration of IgG (g/kg) in colostrum from ewes offered feedblocks was significantly higher than for ewes not offered feedblocks (80 g/kg *v.* 69 g/kg respectively; $P<0.05$). There were no significant difference between treatments in the initial yield of DM, CP, fat, lactose or IgG.

Table 3.9 *Initial concentration (g/kg) and yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Concentration (g/kg)								
DM	348	342	345	378	21.8	NS	NS	NS
CP	186	184	190	202	10.5	NS	NS	NS
Fat	143	130	140	159	13.4	NS	NS	NS
Lactose	25	24	22	22	2.2	NS	NS	NS
Ash	9	15	9	15	3.7	*	NS	NS
IgG	71	68	84	76	0.1	NS	*	NS
Yield (g)								
DM	192.6	196.7	189.3	190.2	35.31	NS	NS	NS
CP	103.8	112.5	95.5	94.9	21.58	NS	NS	NS
Fat	77.3	71.9	64.1	72.4	15.84	NS	NS	NS
Lactose	15.3	16.4	14.2	10.1	3.53	NS	NS	NS
Ash	5.0	11.0	5.4	8.1	3.34	NS	NS	NS
IgG	42.6	38.4	43.7	38.7	6.95	NS	NS	NS

3.3.5.3 Colostrum composition and component yield at 16 hours post partum

Concentrations of DM, CP, fat, lactose, ash and IgG in colostrum at 12-16 hours *post partum* were lower and the concentration of lactose higher than in the initial secretion of colostrum (Table 3.10). There were no significant effects of diet on the 12-16 hour concentration or yield of DM, CP, lactose, ash or IgG. However, there was a tendency for a higher fat (P=0.096) and lower ash (P=0.080) concentration in colostrum produced by ewes offered feedblocks, compared to those fed concentrate alone. In addition, ewes fed concentrates containing fishmeal tended to have a lower 16 hour fat yield in colostrum when the concentrate was fed alone and a higher fat yield when the concentrate was offered with *ad libitum* feedblocks compared to those containing soya-bean meal (P=0.088).

Table 3.10 *Concentration (g/kg) and yield (g/h) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum secreted between 12 and 16 hours post partum by ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Concentration (g/kg)								
DM	227	243	236	257	17.7	NS	NS	NS
CP	73	78	74	81	9.8	NS	NS	NS
Fat	114	113	118	137	11.7	NS	NS	NS
Lactose	41	40	40	38	2.0	NS	NS	NS
Ash	9	12	8	8	1.7	NS	NS	NS
IgG	14	16	12	16	4.4	NS	NS	NS
Yield (g/h)								
DM	27.3	23.2	23.1	27.9	4.20	NS	NS	NS
CP	8.7	7.4	7.4	8.9	1.66	NS	NS	NS
Fat	13.3	10.9	11.7	15.0	2.33	NS	NS	NS
Lactose	4.7	3.7	3.9	3.9	0.57	NS	NS	NS
Ash	1.0	1.0	0.8	0.9	0.18	NS	NS	NS
IgG	1.8	1.7	1.3	1.8	0.53	NS	NS	NS

3.3.5.4 Calculated yield of constituents over the first 24 hours post partum

There were no significant effect of protein source or feedblocks on the calculated 24 hour yield of DM, CP, fat, lactose, or IgG in colostrum (Table 3.11). However, there was a tendency (P=0.076) for ewes offered fishmeal in the concentrate (diets F and FB) to produce a lower yield of ash (g/d) compared to those offered concentrates containing a soya-bean meal based diet (S and SB). In addition, ewes fed concentrates containing fishmeal tended to have a higher DM yield than ewes fed a soya-bean meal based diet when the concentrates were fed alone, but have a lower DM yield compared to ewes fed a soya-bean meal based diet when the concentrates were offered with *ad libitum* feedblocks (P=0.085).

Table 3.11 *Calculated yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) from colostrum secreted during the first 24 hours post partum by ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
DM	798	716	674	856	106.4	NS	NS	NS
CP	303	276	254	300	42.2	NS	NS	NS
Fat	386	331	335	416	61.6	NS	NS	NS
Lactose	123	106	103	104	14.5	NS	NS	NS
Ash	31	36	23	29	6.0	NS	NS	NS
IgG	73	73	70	82	12.0	NS	NS	NS

3.3.6 Litter birth weight and lamb growth rate

The effect of dietary treatment on litter birth weight is presented in Table 3.12. Ewes offered feedblocks had higher litter birth weights than those offered concentrate alone ($P<0.05$) but there was no effect on 42 day litter weight or on the lamb daily live weight gain. Ewes fed concentrate containing soya-bean meal compared to those fed concentrates containing fishmeal tended to produce a higher litter birth weight when the concentrate was fed alone, and a lower litter birth weight when the concentrate was fed with *ad libitum* feedblocks ($P=0.058$). Ewes fed diets containing fishmeal (F and FB) produced lambs with a higher growth rate ($P<0.05$) and 42 day litter weight ($P=0.078$) compared to ewes fed diets containing a soya-bean meal based diet (S and SB).

Table 3.12 *Litter birth weight (kg), 42 day litter weight (kg) and lamb growth rate (g/d) for ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	Treatment means					Significance		
	F	S	FB	SB	s.e.d.	Protein	Blocks	Int
Litter birth weight	8.11	8.46	9.21	8.49	0.386	NS	*	NS
42 day litter weight	34.4	32.3	33.8	30.9	1.92	NS	NS	NS
Lamb growth rate	306	287	295	266	15.6	*	NS	NS

3.3.7 Ewe blood metabolites

Both the mean *pre partum* NEFA and BHB concentrations were significantly lower in ewes fed concentrates containing fishmeal (F and FB; $P<0.05$; Table 3.13). Ewes fed diets containing fishmeal (F and FB) had a mean *pre partum* NEFA concentration of 0.49 mmol/l whilst ewes fed diets containing treated soya-bean meal (S and SB) had a mean concentration of 0.62 mmol/l. In addition, ewes fed diets containing fishmeal (F and FB) tended to have higher *pre partum* glucose concentrations (2.98 mmol/l) compared to those fed diets containing soya-bean

meal (S and SB; 2.84 mmol/l; P=0.080). Ewes offered feedblocks (FB and SB) tended to have higher *pre partum* plasma NEFA concentrations (0.56 *v.* 0.49 mmol/l; P= 0.060) and significantly lower *post partum* plasma BHB concentrations (0.70 *v.* 0.88 mmol/l; P<0.05) compared to those fed concentrate alone. A significant increase in urea-N was seen in ewes offered feedblocks (FB and SB) compared to those not offered feedblocks (F and S) in both the *pre partum* (9.96 *v.* 6.97 mmol/l; P<0.001) and the *post partum* period (11.85 *v.* 9.61 mmol/l; P<0.001). There was no effect of the diet fed on either *pre partum* or *post partum* plasma albumin or total protein concentrations.

Table 3.13 *Pre partum and post partum plasma concentrations of NEFA (mmol/l), BHB (mmol/l), glucose, urea-N (mmol/l), albumin (g/l) and total protein (g/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.*

	F	S	FB	SB	s.e.d	Protein	Blocks	Int
Mean Pre partum:-								
NEFA	0.48	0.50	0.50	0.61	0.045	*	NS	NS
BHB	0.57	0.67	0.55	0.67	0.065	*	NS	NS
Glucose	2.98	2.83	2.97	2.85	0.103	NS	NS	NS
Urea-N	7.29	6.65	9.82	10.10	0.577	NS	***	NS
Albumin	42.1	41.6	42.4	41.8	1.15	NS	NS	NS
Total protein	66.1	65.7	67.8	65.2	1.77	NS	NS	NS
Mean Post partum:-								
NEFA	0.67	0.65	0.75	0.64	0.066	NS	NS	NS
BHB	0.88	0.87	0.65	0.75	0.118	NS	*	NS
Glucose	3.35	3.33	3.28	3.36	0.096	NS	NS	NS
Urea-N	10.22	8.99	11.93	11.76	0.603	NS	***	NS
Albumin	46.8	45.2	45.5	45.7	1.00	NS	NS	NS
Total protein	71.9	72.2	72.0	73.2	2.15	NS	NS	NS

3.3.7.1 Ewe blood metabolic profiles

The mean concentration of plasma NEFA remained relatively constant in ewes fed all treatments from week 6 (overall mean of 0.54 mmol/l) to week 2 (0.55 mmol/l) *pre partum* and subsequently declined towards parturition (0.45 mmol/l at 1 week *pre partum*; Figure 3.7). In lactation, mean plasma NEFA concentration increased from 0.61 mmol/l at 1 week to 0.74 mmol/l at 2 weeks and then subsequently declined to 0.62 mmol/l at 6 weeks of lactation.

Ewes fed concentrates containing fishmeal, compared to those fed concentrates containing a soya-bean based diet, had a lower concentration of plasma NEFA at 1 week *pre partum* (0.41 *v.* 0.48 mmol/l; $P<0.05$). Ewes fed a reduced amount of concentrates and offered feedblocks tended to have a higher plasma NEFA concentration at 1 week *pre partum* (0.49 *v.* 0.40 mmol/l; $P<0.001$) and 1 week post-partum (0.65 *v.* 0.57 mmol/l; $P<0.050$).

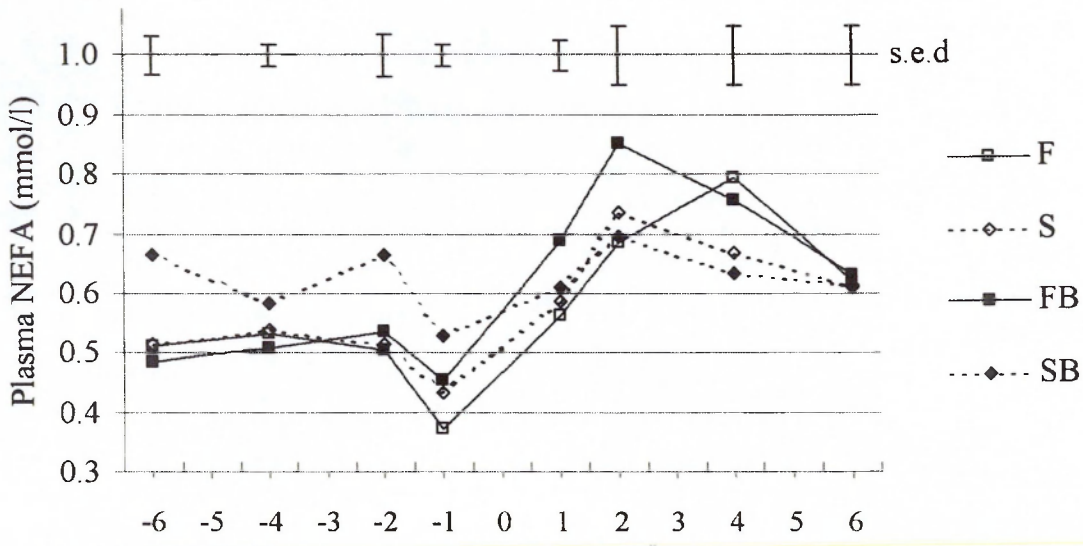

Figure 3.7 Weekly concentrations of plasma NEFA (mmol/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The mean concentration of plasma BHB increased in ewes fed all treatments from 6 weeks (overall mean of 0.39 mmol/l) to 2 weeks (0.83 mmol/l) *pre partum* and subsequently declined towards parturition (0.76 mmol/l at 1 week *pre partum*; Figure 3.8). In lactation, mean plasma BHB concentration increased from 0.70 mmol/l at 1 week to 0.89 mmol/l at 2 weeks *post partum*. Concentrations of plasma BHB were lower at 6 weeks *post partum* compared to 2 weeks *post partum* in ewes fed diets F, FB and SB and were higher at 6, compared to 2 weeks *post partum* in ewes fed diet S.

Ewes fed concentrates containing fishmeal had a lower concentration of plasma BHB compared to ewes fed diets containing a soya-bean based diet at 2 weeks *pre partum* (0.73 *v.* 0.93 mmol/l; $P<0.05$). No effect of dietary protein source on the weekly concentration of plasma BHB occurred in the *post partum* period. At 4 weeks *post partum* ewes fed a reduced amount of concentrates and offered feedblocks had a significantly lower plasma BHB concentration than those offered concentrate alone (0.70 *v.* 0.97 mmol/l; $P<0.05$).

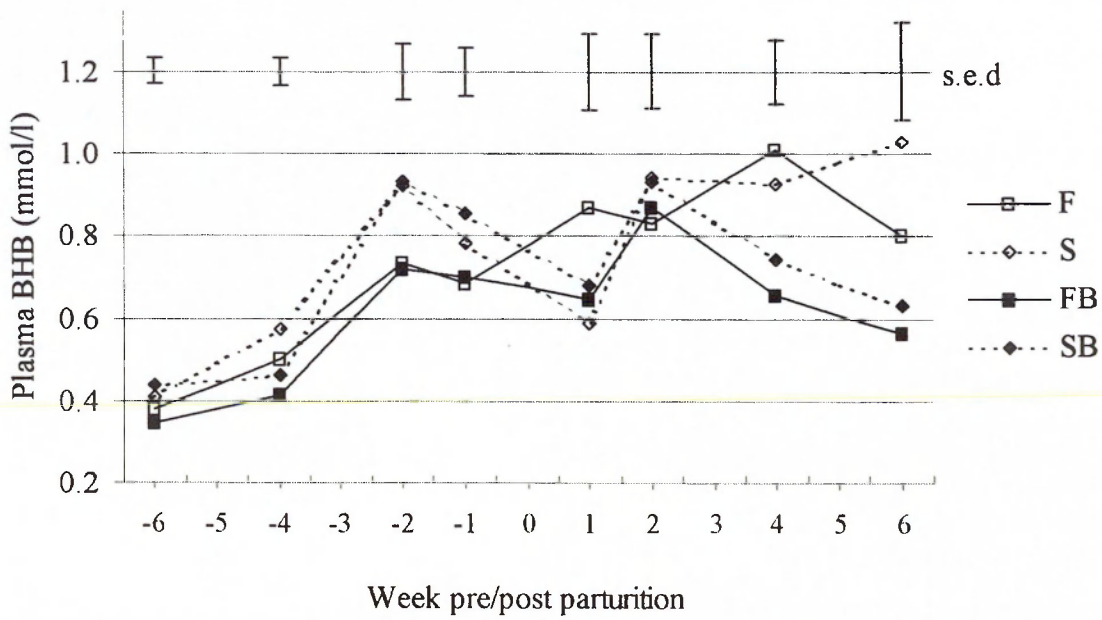

Figure 3.8 Weekly concentrations of plasma BHB (mmol/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The mean concentration of plasma glucose increased in ewes fed all treatments from 6 weeks (overall mean of 2.70 mmol/l) to 1 week (3.16 mmol/l) *pre partum* (Figure 3.9). In lactation, mean plasma NEFA concentration continued to increase in ewes fed all treatments from 3.24 mmol/l at 1 week of lactation to 3.43 mmol/l at 4 weeks and then subsequently declined to 3.27 mmol/l at 6 weeks of lactation.

Ewes fed concentrates containing fishmeal (F and FB) had a significantly higher concentration of plasma glucose compared to ewes fed concentrate containing a soya-bean meal based diet at 1 week *pre partum* (3.30 *v.* 3.03 mmol/l; $P<0.05$). There was no additional effect of dietary treatment on the weekly concentrations of plasma glucose in either the *pre partum* or the *post partum* period.

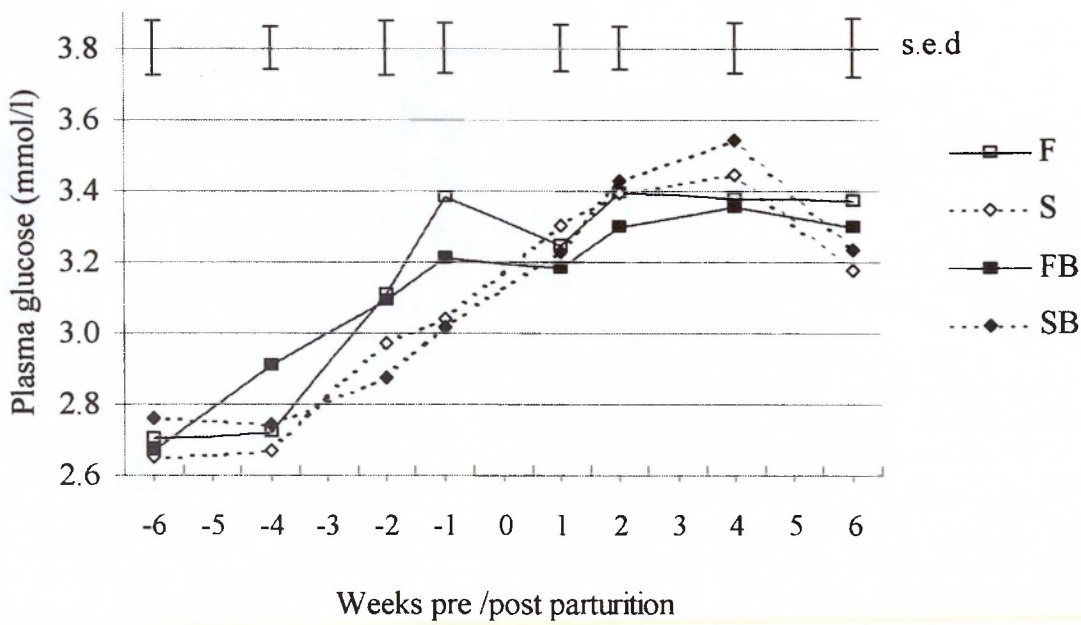

Figure 3.9 Weekly concentrations of plasma glucose (mmol/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The mean concentration of plasma urea-N increased in ewes fed all treatments from 6 weeks (overall mean of 7.7 mmol/l) to 1 week (10.2 mmol/l) *pre partum* (Figure 3.10). In lactation, mean plasma urea-N concentration decreased in ewes fed all treatments from 12.9 mmol/l at week 1 *post partum* to 9.1 mmol/l at week 6.

Ewes fed a reduced amount of concentrates and offered feedblocks (FB and SB) had a significantly higher concentration of plasma urea-N, compared to those not offered feedblocks (F and S) from week 6 to week 1 *pre partum* and at weeks 2, 4 and 6 *post partum* ($P<0.01$). Ewes fed concentrate containing fishmeal (F and FB) had a significantly higher plasma urea-N concentration at 1 week *post partum* compared to those fed diets containing a soya-bean meal based diet (S and SB; $P<0.01$).

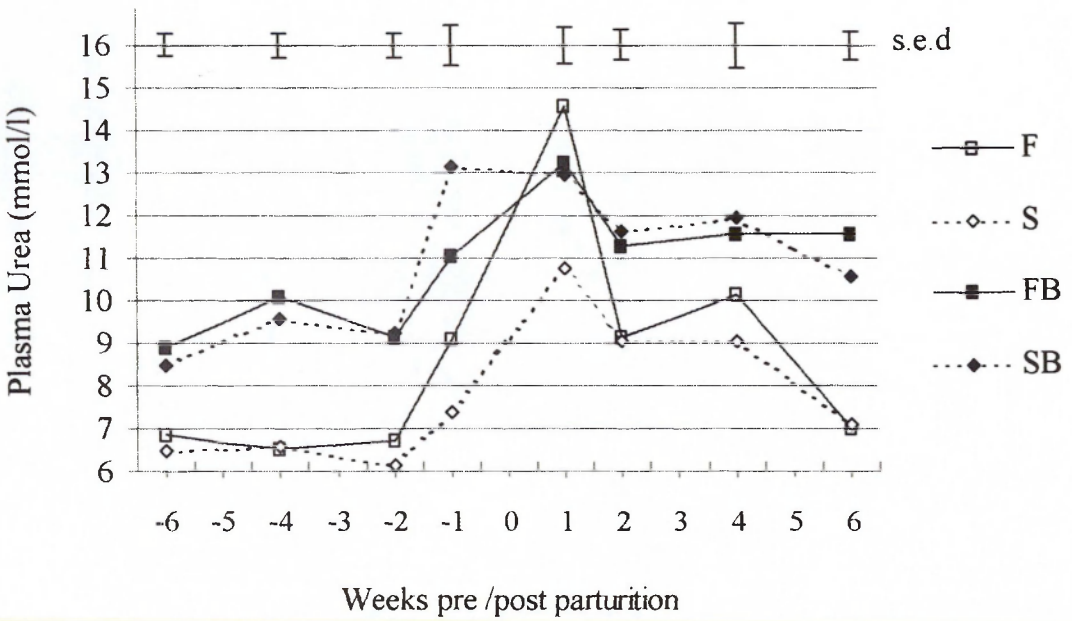

Figure 3.10 Weekly concentrations of plasma urea-N (mmol/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The mean concentration of plasma albumin increased in ewes fed all treatments from 39.4 g/l at week 6 to 47.9 g/l week 1 *pre partum* (Figure 3.11). In lactation, mean plasma albumin concentration decreased in ewes fed all treatments from 50.8 g/l at 1 week *post partum* to 43.4 g/l at 6 weeks.

There was no significant effect of dietary treatment on the concentration of plasma albumin in the *pre partum* period. However, whilst ewes offered feedblocks tended to have a higher plasma albumin concentration at 2 weeks *post partum* compared to those offered concentrate alone (47.2 *v.* 44.3 g/l; $P = 0.070$ respectively) they had a significantly lower plasma albumin concentration at 6 weeks *post partum* (42.1 *v.* 44.7 g/l; $P < 0.05$ respectively).

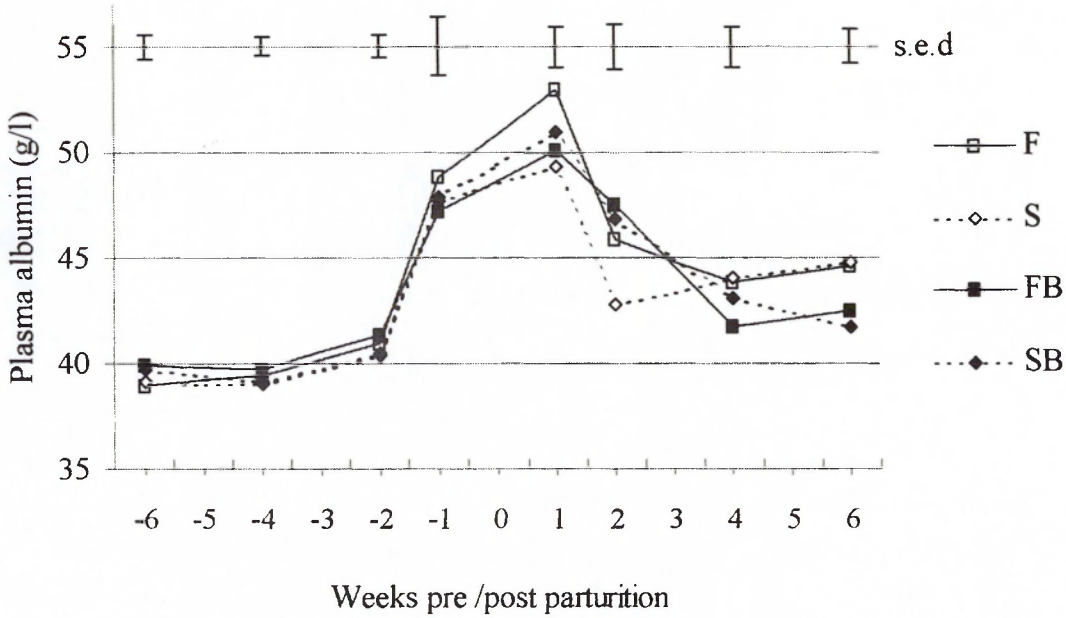

Figure 3.11 Weekly concentrations of plasma albumin (g/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

The mean concentration of plasma total protein decreased in ewes fed all treatments from 66.1 g/l at 6 weeks to 61.1 g/l at 2 weeks *pre partum* and subsequently increased to 75.3 g/l at 1 week *pre partum* (Figure 3.12). In lactation, mean plasma total protein concentration decreased in ewes fed all treatments from 81.1 g/l at 1 week to 67.9 g/l at 6 weeks of lactation.

There was no significant effect of dietary treatment on the concentrations of plasma total protein in the *pre partum* period. Ewes offered feedblocks had a significantly higher plasma total protein concentration at 2 weeks *post partum* (76.6 *v.* 70.4 g/l; $P< 0.05$).

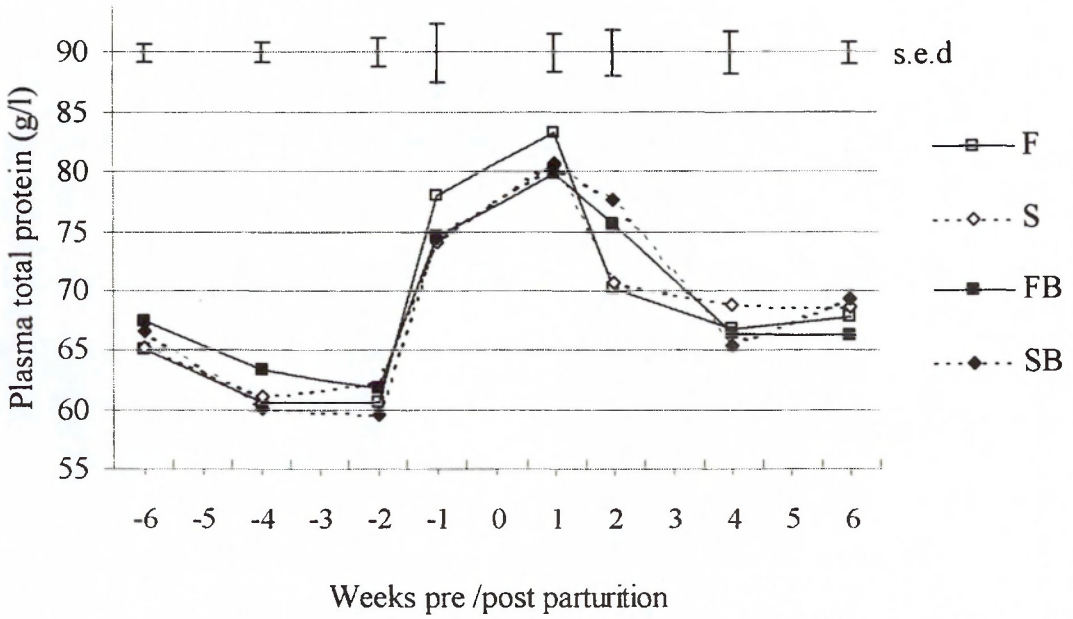

Figure 3.12 Weekly concentrations of plasma total protein (g/l) of ewes fed concentrate containing fishmeal (F) or protected soya-bean meal (S), formulated to be fed alone or with feedblocks (B) to ewes in late pregnancy and early lactation.

3.4 DISCUSSION

3.4.1 Summary of main results

The concentrates containing soya-bean meal (S and SB) had similar degradability coefficients to those containing fishmeal (F and FB) which resulted in a mean MP supply of 129, 118, 138 and 122 g/d during pregnancy and 256, 246, 283 and 256 g/d during lactation for diets F, S, FB and SB respectively. The increase in the *pre partum* intakes of DM, ME, DUP and MP observed when ewes were offered concentrates containing fishmeal (F and FB) compared to those offered a soya-bean meal based diet (S and SB) was caused by an increased straw intake together with a significant increase in the consumption of feedblocks by ewes offered a concentrate containing fishmeal (FB *v.* SB). A higher *post partum* intake of feedblock was also observed on diet FB compared to diet SB, which resulted in increased intakes of ME ($P<0.05$), DUP ($P<0.05$) and MP ($P<0.01$).

3.4.2 Ewe and lamb performance

3.4.2.1 Straw intake

Straw DM intakes were broadly consistent with those seen in other studies with pregnant and lactating sheep (eg. Orr *et al.*, 1985). Ewes on all treatments had a lower straw intake during the *pre partum* period compared to the *post partum* period. Reductions in feed intake during late pregnancy have been well documented by other authors (Robinson, 1987; Everts, 1990), and has largely been attributed to an increase in uterine contents reducing the ability of the rumen to distend (Forbes, 1970).

Intake responses to an increased supply of ERDP have often been observed with straw based rations where intake is limited by the rate of fibre digestion (Newbold, 1994). Since urea accounts for approximately 61% of CP within feedblocks and as urea supplies entirely rumen

degradable nitrogen (AFRC, 1993), an increase in straw intake may be expected when offering feedblocks (Newbold, 1994). However, in the current experiment, offering feedblocks did not affect barley straw DM intake. Although significantly higher levels of plasma urea-N were observed both *pre partum* and *post partum* in ewes offered feedblocks (FB and SB), high blood urea-N values were observed on all diets and in the absence of a measured rumen ammonia-N concentration it may be suggested that rumen ammonia-N was adequate for maximum fibre digestion on all diets (Topps and Thompson, 1984). Any further increase in the supply of degradable protein from the feedblocks would therefore be unlikely to result in improvements in rumen fibre degradation, rumen outflow rate or DM intake. Kendall (1977) reported that increased proximity of stock to feedblocks tends to enhance the rate of intake of the feedblock and in the current experiment the individual penning of ewes may have led to the high intakes of feedblock observed. Ewes in this situation were in close confinement to the feedblock and had no competition for it.

3.4.2.2 Colostrum production

Colostrum yield and subsequent secretion rates on all treatments were in agreement with figures quoted by other authors for similar lowland breeds (Mellor and Murray, 1986; Pattinson and Thomas, 1998). Robinson (1987) presented unpublished data by McPherson *et al.* (1981) which demonstrated that colostrum yield responds positively to the daily CP intake by ewes in late pregnancy, particularly when ME intakes are low and the supplementary protein source is relatively undegradable in the rumen. However, in agreement with the current results, Dawson *et al.* (1999), found no difference in the yield of colostrum when ewes were fed concentrates containing similar levels of DUP, but containing either fishmeal or xylose treated soya-bean meal (Soypass™, Borregaard) as the main protein source.

Colostrum yield, constituent concentration and constituent yield are particularly variable between individual sheep (Pattinson *et al.*, 1995) which therefore makes it difficult to detect any differences due to dietary treatment. Hall and Egan (1988) recorded 1 hour colostrum yields ranging from less than 50 g per ewe to over 2 kg, whilst O'Doherty and Crosby (1997) found coefficients of variation of 74, 44 and 42 % for yields of colostrum at 1 hour, 10 hour and 18 hours *post partum* respectively. Large coefficients of variation of 64 and 47 % were also found in the current work for the initial yield and the secretion rate at 12 - 16 hours *post partum* respectively. This may account for the apparent lack of difference observed in the current experiment in the colostrum yield, constituent concentration and constituent yield at birth and the secretion rate at 12 - 16 hours *post partum*.

In the current experiment, the concentration of IgG in colostrum was higher in ewes fed a reduced amount of concentrate and feedblocks. Increases in the IgG concentration in colostrum have been observed by other authors when nutrient intake is increased (O'Doherty and Crosby, 1997) and in the current experiment this may be attributed to an increase in nutrient supply from the higher than expected feedblock intake. O'Doherty and Crosby (1997) observed increases in the IgG concentration in colostrum at 1, 10 and 18 hours *post partum* when CP intake was increased from 76 to 142 g/day or when supplements of molassed sugar beet pulp were given in addition to grass silage from day 91 of pregnancy through to lambing.

O'Doherty and Crosby (1997) and Pattinson *et al.* (1991 and 1995) observed a negative linear relationship between the yield of colostrum at 1 hour *post partum* and the IgG concentration in colostrum. In the current experiment, a negative correlation between the initial yield and the initial concentration of IgG was also observed ($P<0.001$; $r^2 = 33.0$). The higher IgG concentration observed on diets FB and SB may simply be in response to the lower mean yields

of colostrum measured. There was no effect of offering feedblocks on the total yield of IgG.

3.4.2.3 Litter birth weight

The mean litter birth weight observed in this study was 8.57 kg and is comparable with other studies involving twin-bearing, lowland ewes. Hill and Notman (1998), Pattinson and Thomas (1998), Dawson *et al.* (1999) and O'Doherty and Crosby (1997) reported mean litter birth in twin-bearing lowland ewes weights of 8.57, 8.30, 8.57 and 8.82 kg respectively.

Diets used in the current experiment that were formulated to contain either fishmeal or protected soya-bean meal, had no effect on lamb birth weight. The results from the current experiment were in agreement with those of Dawson *et al.* (1999), who found that feeding pregnant ewes concentrates that were formulated to supply similar levels of DUP, but containing either fishmeal or soypass as the main protein source had no effect on lamb birth weight. In addition, in the current experiment, diets provided ME intakes close to the ewes requirement and it would appear that a maternal energy deficit is necessary in order to elicit a birth weight response to proteins that are less degradable in the rumen. Even where the main protein sources compared were urea or the less degradable fishmeal, Dawson *et al.* (1999) found no difference in lamb birth weight of ewes fed close to their energy requirements.

In contrast, offering feedblocks to ewes did increase litter birth weight and it is likely to be a response to higher than expected consumption of feedblocks leading to increases in calculated *pre partum* ME intake. Ducker *et al.* (1981) reported intakes of feedblocks ranging from 100 to 472 g/d under hill and upland conditions. In the current experiment, diets were formulated at an expected intake figure 450 g/day of feedblock. The figure chosen was at the upper end of those quoted by Ducker *et al.* (1981) as increases in feedblock intake were observed by

Kendall (1977) when the feedblock was placed in close proximity to the ewe. In the current experiment, ewes offered feedblocks were fed concentrates at 0.75 of those offered concentrates alone as this proportion would provide an equal ME intake at the expected feedblock consumption. However, the actual mean intake of feedblock was 491 g/day. Given the large weight gains that the foetuses make during the final 6 weeks of pregnancy (Robinson, 1983b) it is reasonable to expect that lamb birth weight would respond to the extra ME consumed (Robinson, 1983a; Rattray, 1992). Despite the increase in calculated *pre partum* ME intake in ewes offered feedblocks compared to those not offered feedblocks, no significant difference in plasma glucose concentration was seen in the current experiment. Glucose is the major energy substrate for the developing foetus (Russel *et al.* 1967b) and given that feedblocks increased litter birth weight, the lack of any difference may be due to increased utilisation of glucose by the developing foetuses. Russel and Wright (1983), in a comparison of blood metabolites as indicators of energy status, reported that plasma glucose was the least satisfactory index of energy status in pregnant and lactating ewes. The use of glucose as an index for energy status has limitations as it is homoeostatically controlled and quickly responds to adrenal cortical hyperactivity and could account for the absence of significant differences in either *pre partum* or *post partum* concentrations of plasma glucose in the current experiment. Differences in plasma glucose have only been reported when ewes are undernourished for prolonged periods (Everts, 1990; Petterson *et al.*, 1994; Hussain *et al.*, 1996) and in the current study ewes on all treatments had plasma glucose concentrations well in excess of the 1.66 mmol/l reported by O'Doherty and Crosby (1998) required to predispose the ewe to hypoglycaemia.

3.4.2.4 Lamb growth rate, ewe weight and condition score change

Changes in ewe weight *pre partum* and *post partum* were similar to values reported by other

authors for lowland breeds (Chapple *et al.*, 1998; Pattinson and Thomas, 1998). However, ewe weight change has been cited as a poor indicator of nutritional adequacy, particularly in late pregnancy due to the relative changes in the weight of the maternal body and of the intra-uterine contents and led authors to use body condition scoring as a measure of nutritional adequacy (Russel *et al.* 1969).

The ability of fishmeal to stimulate mobilisation of body fat is well documented (Gonzales *et al.*, 1982; Robinson, 1987) and is often attributed to the increase in non-ammonia nitrogen reaching the small intestine when compared to feeding protein sources of high rumen degradability (Ngongoni *et al.*, 1989). In addition, it is well established that mobilisation of adipose tissues results in elevated plasma concentrations of free fatty acids or NEFA (Annison, 1960; Patterson *et al.*, 1964). In the current experiment, both mean plasma NEFA concentrations and the rate of condition score loss were higher during lactation than in pregnancy. When condition score change and plasma NEFA concentrations are considered together, feeding diets containing fishmeal (F and FB) appeared to increase the deposition of adipose tissue in the *pre partum* period and increase the rate of adipose loss during lactation when compared to ewes fed concentrate containing soya-bean meal based diet (S and SB). The increase in non-ammonia nitrogen will elicit a higher milk yield (Gonzalez *et al.*, 1982), but only in conjunction with increases in ME supply. In the current experiment, as there was no significant difference in straw intake between ewes fed any of the dietary treatments, the extra ME could only come from increases in mobilised body fat, providing the ewe is in adequate body condition (>2.5; Robinson, 1987 and 1990). In this experiment, the diets compared were designed to supply the same level of MP *post partum*. However, diet F, S, FB and SB supplied 256, 246, 283 and 256 gMP/d respectively. The increase in *post partum* body condition loss observed in ewes fed diets containing fishmeal (F and FB) could therefore be explained by the

increase in MP supply.

Mean plasma concentrations of BHB were lower during pregnancy compared to lactation. This result is in agreement with both the lower plasma NEFA concentrations observed in pregnant compared to lactating ewes and with the increases in condition score observed during late pregnancy and the subsequent loss of condition recorded during lactation. In addition, plasma BHB concentration was also lower in the *pre partum* period for ewes fed concentrates containing fishmeal (F and FB) than for ewes fed concentrates containing a soya-bean meal based diet (S and SB). Plasma BHB concentrations increase when low glucose concentrations limit its metabolism via the TCA cycle due to insufficient concentrations of oxaloacetate (Payne, 1989). Significantly higher MP intake in ewes fed diets containing fishmeal (F and FB) may have provided additional amino acids to be used for gluconeogenesis, reducing the concentration of BHB in the plasma. Offering feedblocks during lactation also significantly reduced plasma BHB concentration. As high concentrations of BHB are associated with energy deficits (O'Doherty and Crosby, 1998) it is likely that the higher feedblock consumption than expected reduced plasma BHB concentration by reducing the energy deficit. Russel (1984) deemed BHB to be the most suitable indicator of energy status in a wide range of situations.

In addition to the extent of protein degradation in the rumen, the digestibility of DUP in the small intestine and the amino acid profile of the DUP is also very important and should ideally complement the profile of the bacterial protein leaving the rumen to match the requirements of the productive animal. As well as being a source of DUP, fishmeal has often been cited as having an appropriate balance of amino acids for lactating ewes (Robinson, 1987). An appropriate balance of amino acids in the diet may elicit a higher rate of body fat loss and in turn may improved milk yield. It is widely accepted that methionine is one of the first limiting

amino acids in soya-bean meal for lactating ruminants (Lynch *et al.*, 1991; Baldwin *et al.*, 1993 and Rodehutscord *et al.*, 1999) and in an attempt to rectify this 0.75 g/kg of a protected methionine (Smartamine™, Rhône Poulenc) was added to diets S and SB. However, the concentrates containing treated soya-bean meal (S and SB) still failed to produce the same level of body fat mobilisation as the concentrates containing fishmeal and it is possible that amino acids other than methionine were limiting the rate of body fat mobilisation. Broderick *et al.* (1974), Clark (1975), Schwab *et al.* (1976) and Lynch *et al.* (1991) concluded that lysine was either co-limiting or soon became limiting after supplementation of diets with methionine for lactating ewes. Thus, in this experiment, it is possible that lysine was limiting any increase in body fat mobilisation on diets S and SB.

Ewes fed concentrates containing fishmeal (F and FB), in addition to an increased rate of condition loss, had lambs with higher liveweight gains from birth to 6 weeks *post partum* than those fed concentrates containing treated soya-bean meal. The increased rate of body fat loss in lactation for ewes on treatments F and FB was presumably contributing to an increased milk yield, although milk production or quality was not assessed. However, it is widely accepted that the main contributing factor to differences in lamb growth during the first 6 weeks *post partum* is milk yield (Doney *et al.*, 1981).

3.6 CONCLUSIONS

Acceptable levels of production can be achieved by replacing fishmeal with formaldehyde protected soya-bean meal protein with an improved amino acid balance in concentrates for pregnant and lactating ewes. Feedblocks can partially replace concentrates for ewes in late pregnancy and early lactation.

CHAPTER 4

THE EFFECTS OF SOURCE AND FORMALDEHYDE TREATMENT OF DIETARY PROTEIN ON THE METABOLISM AND PERFORMANCE OF HOUSED, STRAW FED, PREGNANT AND LACTATING EWES

4.1 INTRODUCTION

The majority of experiments designed to investigate the use of formaldehyde for treating vegetable protein sources have mainly been concerned with the efficiency with which treatment reduces rumen N degradability and the effect on the subsequent availability of rumen undegradable protein in the small intestine (Barry, 1976; Mir *et al.*, 1984; Subuh *et al.*, 1994). Less work has been conducted on the effects of formaldehyde treatment of protein sources on the subsequent performance of pregnant and lactating ewes. In addition, the work that has been conducted has concentrated on the effects of feeding formaldehyde treated soya-bean meal to ewes (Dawson *et al.*, 1999). The experiment reported in Chapter 3 demonstrated that is possible to replace fishmeal with a product based on formaldehyde treated soya-bean meal in diets fed to ewes in late pregnancy and early lactation. However, soya-bean meal as a potential alternative to fishmeal has the distinct disadvantage that it is not possible to grow large quantities of the soya-bean in the United Kingdom and therefore importing large quantities has obvious consequences on the United Kingdom balance of trade.

Field beans and rapeseed meal are both important UK produced protein sources. In 1998, there were 110 000 ha of field beans and 505 000 ha of oilseed rape grown, yielding 3.0 and 3.4 t/ha respectively (Cottle and Cottle, 1999). The area of field beans and oilseed rape grown is comparatively large compared with other UK protein crops, for example, there are only 90 000

ha of linseed and 96 000 ha of dried peas grown (Cottle and Cottle, 1999). Both rapeseed meal and field beans are important as a break for cereals in a crop rotation (Cottle and Cottle, 1999). In addition, the use of oilseed rape is likely to increase in the future as developed nations look for alternative sources of oil for industrial use (Williamson and Badr, 1998), whilst the use of legumes, such as beans, may have a role in reducing reliance on artificial nitrogen fertilizers (Oyer and Touchton, 1990). Similar gross margins can be expected by growing oilseed rape and field beans as other important UK crops such as winter wheat and winter barley (Nix, 2001).

The current study was designed to investigate the effects of including rapeseed meal or field beans, either untreated or formaldehyde treated in concentrates for housed, straw fed, pregnant and lactating ewes as an alternative to including fishmeal.

4.2 MATERIAL AND METHODS

4.2.1 Animals

At 103 days of gestation, 60 twin bearing Charollais x Lleyn (n=10), Charollais x Cambridge (n=10) and Friesland x Lleyn (n=40) ewes were randomly allocated to one of five dietary treatments by breed, weight and condition score. All animals used in this experiment were mature ewes, between 3 and 6 years of age and in lamb to Charollais rams.

4.2.2 Diets

The five dietary treatments differed in the main protein source contained in the concentrate. These were fishmeal (F; 71 g/kg), rapeseed meal (RSM; 119 g/kg), field beans (FB; 164 g/kg), formaldehyde treated rapeseed meal (fRSM; 119 g/kg) or formaldehyde treated field beans (fFB; 164 g/kg). Formaldehyde treatment was carried out by applying 7.9 litres of a 30 % formaldehyde solution to one tonne of field beans (FB; 2.38 g formaldehyde per kg of test protein) or by applying 12.7 litres of a 30 % formaldehyde solution to one tonne of rapeseed meal (RSM; 3.80 g formaldehyde per kg of test protein). The test proteins were formulated to supply 50 g/kg DM of crude protein, or approximately 25 % of the total CP content of the concentrate. The inclusion rate of treated and untreated protein sources were the same for each protein type. Concentrates were formulated to be isoenergetic (13 MJ ME/kg DM) and isonitrogenous (206 g CP/kg DM) and to have an ERDP:FME ratio of >11.5 g/MJ (Table 4.1). All concentrates were supplied on the same incremental scale (Table 4.2) and at a rate which would meet the MP requirements for a ewe on the rapeseed meal (RSM) and field bean (FB) treatments according to AFRC (1992). The experiment ran from six weeks prior, to four weeks post lambing.

Table 4.1 *Dietary composition (g/kg) and predicted chemical composition (g/kgDM) of concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) fed to ewes during late pregnancy and early lactation*

	Treatment				
	F	RSM	fRSM	FB	fFB
Ingredient (g/kg)					
Molasses	100	100	100	100	100
Barley	693	628	628	583	583
Sugar beet pellets (molassed)	50	50	50	50	50
Fishmeal	71				
Field beans				164	164
Rape seed meal		119	119		
Minerals / vitamins	30	30	30	30	30
Urea	11	14	14	14	14
Megalac	15	20	20	20	20
Soya bean meal	30	40	40	40	40
ME (MJ/kgDM)	13.0	12.8	12.8	13.0	13.0
CP	206	206	206	206	206
EE	35	36	36	34	34
NDF	158	183	183	166	166
FME (MJ/kgDM)	11.8	11.6	11.6	11.8	11.8
ERDP[1]	141	156	138	159	137
ERDP[2]	132	148	128	150	126
DUP[1]	40	24	43	23	45
DUP[2]	48	32	48	31	50

[1] calculated at a rumen outflow rate (r) = 0.05

[2] calculated at a rumen outflow rate (r) = 0.08

Table 4.2 *Amount of concentrate (kg fresh weight/ewe/day) containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) fed to ewes during late pregnancy and early lactation*

Weeks *pre/post partum*	Concentrate (kg/d)
-6 to -4	0.9
-4 to-3	1.0
-3 to -2	1.1
-2 to -1	1.2
-1 to 0	1.3
+0 to +4	1.8

4.2.3 Procedure and measurements

At 6 weeks *pre partum*, ewes which had conceived to the first oestrus after sponge withdrawal (n=40) were individually penned and bedded on sawdust, whilst those conceiving to the second oestrus were housed and bedded on straw in one of five group pens. All ewes were offered straw daily as a single feed at 0800 hours at proportionally 1.25 of the previous calculated intake. Straw refusals were removed from individually penned ewes and weighed (±10 g) at 0730 hours on Mondays, Wednesdays and Fridays. Straw offered was sampled weekly (Wednesday) by taking 6 equal samples of straw from six separate bales and a 200 g sample of refused straw was taken from individually penned sheep on Mondays. Concentrates were fed in 2 equal meals (0830 and 1630 hours) up to three weeks *pre partum* and in three equal meals (0800, 1300 and 1630 hours) thereafter. Concentrates were sampled weekly (Wednesday) by taking equal amounts (200 g) from four separate 25 kg bags. All samples of straw and concentrate were stored at 4°C in airtight containers until subsequent analysis.

Ewe liveweight, body condition score, lamb birth weight, lamb weekly weight, colostrum production and milk production were measured. Blood samples were taken at 10 am at weeks 6, 4, 2 and 1 *pre partum* and at 1, 2, and 4 weeks of lactation by the method described in Chapter 2.

Samples of colostrum were analysed for DM, fat, CP, IgG, lactose and ash, whilst samples of ewes milk were analysed for; total solids, fat, CP and lactose (Chapter 2). Blood plasma samples were analysed for total protein, albumin, urea nitrogen, BHB, NEFA and glucose as described in Chapter 2.

4.2.4 The *in-situ* rumen degradability of nitrogen

The *in-situ* nitrogen degradability in the five concentrate feeds was measured using four rumen cannulated sheep.

4.2.4.1 Experimental animals, treatment and design

Four wether sheep aged 7 years with an average weight of 85 kg (s.d. 1.8 kg) and fitted with permanent rumen cannulae of 39 mm internal diameter, were assigned to an incomplete 4 x 4 latin square design and housed in individual, slatted floor pens, with free access to water and mineral licks. Animals were introduced to their surroundings and trial diet two weeks prior to the insertion of polysynthetic fibre bags.

A basal concentrate diet was formulated to be a mean of the diets used in the production trial (Table 4.3).

Table 4.3 *Dietary composition (g/kg) of the basal concentrate fed to the rumen-cannulated wethers*

	Composition (g/kg)
Ingredient	
Barley	641
Molasses	103
Sugar beet pellets (molassed)	50
Fishmeal	15
Field beans	34
Formaldehyde treated field beans	34
Rapeseed meal	25
Formaldehyde treated rapeseed meal	25
Soya-bean meal	39
Urea	14
Megalac	20

The wethers were then offered barley straw to achieve a diet with a ratio of barley straw:concentrate of 0.43:1, which was the same as the ratio consumed by the ewes in the production experiment. Concentrates were offered as a coarse mix in two equal feeds at 0830 and 1630 hours and straw at 0835 and 1635 hours. Diets were fed at 1.1 x maintenance requirements (AFRC, 1993).

Samples were collected and processed as described in Chapter 2.

4.2.5 Statistical analysis

The experiment was designed as a 2 x 2 factorial design with main effects of protein source (FB *v.* RSM) and formaldehyde treatment (with or without formaldehyde) with an additional control containing fishmeal (F). Treatment differences in litter birth weight and growth rates were analysed using number of males in the litter as a co-variate. Lamb growth rate was estimated by linear regression. All statistical analyses was performed by analysis of variance (ANOVA) using Genstat 5 release 3.2 (Lawes Agricultural Trust, 1995).

4.3 RESULTS

The data from two ewes was excluded from the results. Of these, one ewe (diet F) aborted as a result of an unknown cause, prior to the expected lambing date, and one died (diet RSM) in the first week of lactation. Lactation data (except colostrum) for two further ewes was excluded from the results. These ewes (one on each of diets fRSM and fFB) reared only one lamb, the other being born dead.

4.3.1 Diet composition

The determined chemical composition of the concentrates and straw are shown in Table 4.4. The chemical composition of all five concentrates was similar and close to that predicted, with mean values for DM of 846 g/kg, CP 199 g/kg, EE 35 g/kg DM, ash 74 g/kg DM, NDF 174 g/kg DM, ADF 70 g/kg and ADIN 1.20 g/kg DM. However, higher concentrations of CP were observed in the concentrates containing rapeseed meal (RSM and fRSM; 208 g/kg DM) compared to the concentrates containing field beans (FB and fFB; 199 g/kg DM). The chemical composition of the fresh and refused straw were both similar (Table 4.4).

Table 4.4 *Determined chemical composition (g/kg DM) of concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) and determined chemical composition (g/kgDM) straw offered to and refused by ewes during late pregnancy and early lactation*

	F	RSM	fRSM	FB	fFB	Straw	Refused straw
g/kg DM							
DM (g/kg)	854	855	848	836	836	860	880
CP	198	211	204	192	192	33	29
EE	37	35	35	34	34	8	6
Ash	82	75	71	72	66	43	46
NDF	160	175	199	169	169	755	702
ADF	56	77	79	68	70	462	495
ADIN	0.93	1.44	1.56	1.01	1.07	1.2	1.4

4.3.2 Nitrogen degradability of the concentrates

Nitrogen degradability coefficients of the concentrates are presented in Table 4.5. The concentrate containing fishmeal (F) had a high readily soluble (*a*) fraction and a high rate of N degradation (*c*), of the potentially degradable fraction (*b*). Formaldehyde treatment of rapeseed meal resulted in a lower soluble N fraction (*a*) (0.43 *v.* 0.35 for diets RSM and fRSM respectively; $P<0.001$) and in a lower rate of N degradation (*c*) (0.136 *v.* 0.107 for diets RSM and fRSM respectively; $P<0.001$) of the potentially degradable N fraction (*b*). Formaldehyde treatment of field beans also resulted in a lower soluble N fraction (*a*) (0.32 *v.* 0.28 for diets FB and fFB respectively; $P<0.001$) and in a lower rate of degradation (*c*; 0.310 *v.* 0.103 for diets FB and fFB respectively; $P<0.001$) of the potentially degradable N fraction (*b*), with the decrease in rate of N degradation (*c*) of the potentially degradable N fraction (*b*) being greater in FB than RSM. At a rumen outflow rate of 0.05 h^{-1}, formaldehyde treatment reduced the calculated effective N degradability of diets containing rapeseed meal from 0.80 to 0.73 (RSM and fRSM respectively; $P<0.001$) and for diets containing field beans from 0.85 to 0.70 (FB and fFB respectively; $P<0.001$). The effective degradability of concentrate containing fishmeal is more similar to concentrates containing untreated vegetable protein sources (RSM and FB) than those containing formaldehyde treated vegetable protein sources (fRSM and fFB).

Table 4.5 *Nitrogen degradability coefficients for concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) fed to ewes during late pregnancy and early lactation*

	F	RSM	fRSM	FB	fFB	s.e.d.
a	0.50	0.43	0.35	0.32	0.28	0.008
b	0.39	0.51	0.56	0.62	0.63	0.002
c	0.183	0.136	0.107	0.310	0.103	0.0130
a+b	0.89	0.94	0.91	0.94	0.91	0.004
r^2	97.0	95.8	95.5	96.2	93.7	
Effective N degradability (P):-						
r = 0.05	0.81	0.80	0.73	0.85	0.70	0.014
r = 0.08	0.77	0.75	0.67	0.81	0.63	0.014

Where *a* is the immediately soluble fraction, *b* is the insoluble but potentially degradable fraction, *c* is the constant rate of degradation of *b* and r is the rumen outflow rate per hour. Effective N degradability (P) was calculated according to the equations given in Chapter 2.

4.3.3 Feed and nutrient intake

The effect of protein source and formaldehyde treatment on the intakes of concentrate, straw, DM, ME, DUP and MP in the *pre partum* and *post partum* period is presented in Table 4.6. Weekly intake of straw, DM, ME, DUP and MP is presented in Figures 4.1, 4.2, 4.3, 4.4 and 4.5 respectively. In both the *pre partum* and *post partum* periods all ewes consumed their total concentrate allocation and therefore concentrate intake was not affected by treatment.

There was no significant effect of treatment on the intakes of straw, DM or ME by ewes during the *pre partum* period. However, ewes fed formaldehyde treated protein had a significantly higher *pre partum* DUP intakes (48 *v.* 32 g/d respectively; $P<0.001$) and MP intake (130 *v.* 116 g/d respectively; $P<0.001$) than those fed untreated protein. In addition, ewes fed diets containing rapeseed meal (RSM and fRSM) also had a higher *pre partum* DUP intake (43 *v.* 38 g/d respectively; $P<0.001$) and MP intakes (127 *v.* 120 g/d respectively; $P<0.05$) than those ewes fed the concentrates containing field beans (FB and fFB). Ewes fed the concentrate containing fishmeal had significantly lower DUP intakes than the mean of ewes fed the other treatments (36 *v.* 40 g/d; $P<0.01$). There was a significant interaction in *pre partum* DUP supply ($P<0.001$), with formaldehyde treatment of protein increasing the calculated DUP supply for ewes fed concentrates containing field beans (FB *v.* fFB; 27 *v.* 48 g/d) to a greater extent than in ewes fed concentrates containing rapeseed meal (RSM *v.* fRSM; 37 *v.* 49 g/d).

In the *post partum* period, ewes fed the concentrate containing fishmeal (F) had a significantly higher intake of ME (23.5 *v.* 22.8 MJ/day respectively; $P<0.05$) and DUP (69 *v.* 80 g/day respectively; $P<0.01$) than the mean of the ewes fed other diets, and tended to have higher intakes of straw (0.57 *v.* 0.47 kg DM/day respectively; $P= 0.092$) and of total DM (2.10 *v.* 2.00 kg DM/day respectively; $P= 0.092$). Ewes fed concentrates treated with formaldehyde (fRSM

and fFB *v.* RSM and FB), had higher intakes of straw (0.54 *v.* 0.41 kg DM/day respectively; $P<0.05$), DM (2.07 *v.* 1.94 kg/day respectively; $P<0.05$), ME (23.2 *v.* 22.4 MJ/day respectively; $P<0.05$) and DUP (95 *v.* 65 g/day respectively; $P<0.01$) during the *post partum* period. There was a significant interaction in both *post partum* DUP ($P<0.001$) and MP intake ($P<0.05$), with formaldehyde treatment of protein increasing the calculated DUP and MP supply for ewes fed concentrates containing field beans (53 *v.* 101 g/d DUP; 186 *v.* 202 g/d MP for FB *v.* fFB respectively) to a greater extent than in ewes fed concentrates containing rapeseed meal (77 *v.* 89 g/d DUP; 200 *v.* 204 g/d MP for RSM *v.* fRSM respectively).

Table 4.6 *Intake of concentrate (kg DM/d), straw (kgDM/d), total dry matter (DM; kg/d)and calculated intake of metabolisable energy (ME; MJ/d) digestible undegradable protein (DUP) and metabolisable protein (MP; g/d) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Pre partum:-										
Concentrate	0.91	0.91	0.91	0.91	0.91	-	-	-	-	-
Straw	0.54	0.47	0.59	0.50	0.46	0.066	NS	NS	NS	NS
DM	1.45	1.37	1.49	1.41	1.37	0.066	NS	NS	NS	NS
ME	15.2	14.6	15.4	15.0	14.7	0.42	NS	NS	NS	NS
DUP	36	37	49	27	48	1.6	**	***	***	***
MP	123	119	134	112	127	3.5	NS	*	***	NS
Post partum:-										
Concentrate	1.53	1.53	1.53	1.53	1.53	-	-	-	-	-
Straw	0.57	0.39	0.50	0.43	0.57	0.069	NS	NS	*	NS
DM	2.10	1.92	2.03	1.96	2.10	0.069	NS	NS	*	NS
ME	23.5	22.1	22.9	22.6	23.5	0.44	*	NS	*	NS
DUP	69	77	89	53	101	4.2	**	***	NS	***
MP	200	204	206	186	202	4.0	NS	***	**	*

4.3.3.1 Intake of concentrate, straw, dry matter, metabolisable energy and metabolisable protein

The intake of straw increased in ewes on all of the treatments from week 6 (overall mean of 0.44 kg DM/ewe/day) to week 3 (0.58 kg DM/ewe/day) *pre partum* and subsequently declined towards parturition (0.43 kg DM/ewe/day; Figure 4.1). Straw intakes subsequently increased as lactation progressed, with ewes eating 0.66 kg DM/ewe/day on average during the fourth week of lactation. No significant effect of treatment on the straw intake of ewes was observed during any week of pregnancy. However, in the *post partum* period, ewes fed diets containing formaldehyde treated protein sources (fRSM and fFB) had significantly higher straw intakes than those fed untreated protein sources (RSM and FB) during all weeks of lactation ($P<0.05$).

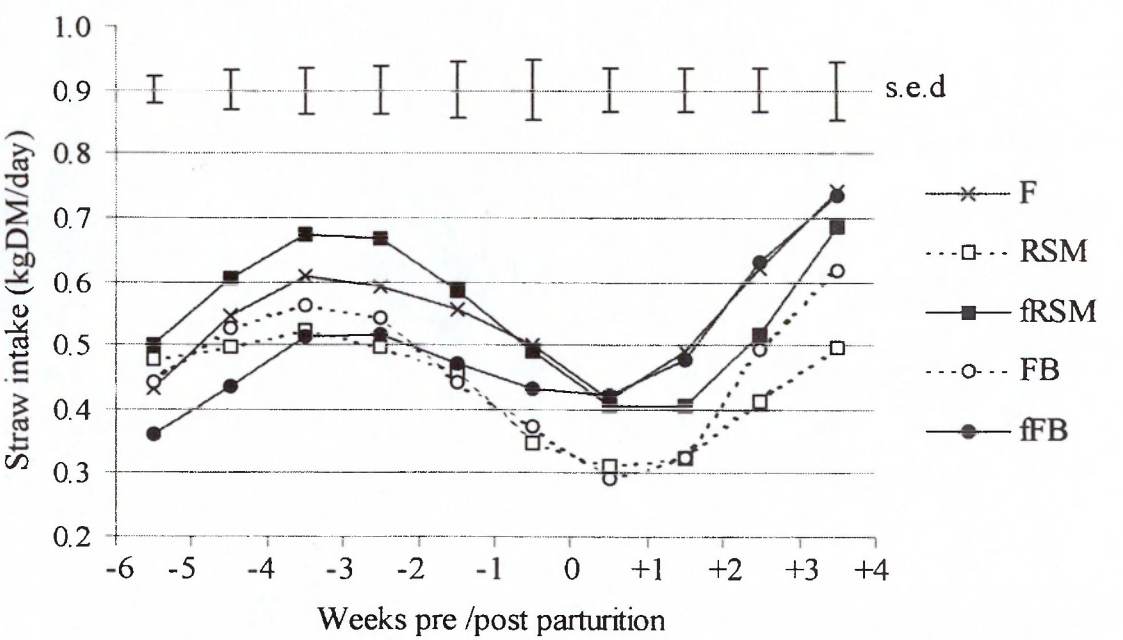

Figure 4.1 Intake of straw (kgDM/day) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

Total DM intake (kg/ewe/day; Figure 4.2) increased on all treatments from 6 to 3 weeks *pre partum* as a result of increasing concentrate fed from 0.9 kg fresh weight/ewe/day at 6 weeks *pre partum* to 1.1 kg fresh weight/ewe/day at 3 weeks *pre partum* and as a result of the increasing straw intake. From 3 weeks *pre partum* to parturition no further increase in dry matter intake was seen in ewes fed diets RSM, fRSM or FB, and only small increases occurred in ewes fed diets F and fFB. After parturition, the DM intake by ewes was increased. No significant effect of treatment on the DM intake of ewes *pre partum* was observed during any week of pregnancy. However, in the *post partum* period, ewes fed diets containing formaldehyde-treated protein sources had significantly higher intakes of DM compared to those fed untreated protein sources in all weeks of lactation ($P<0.05$).

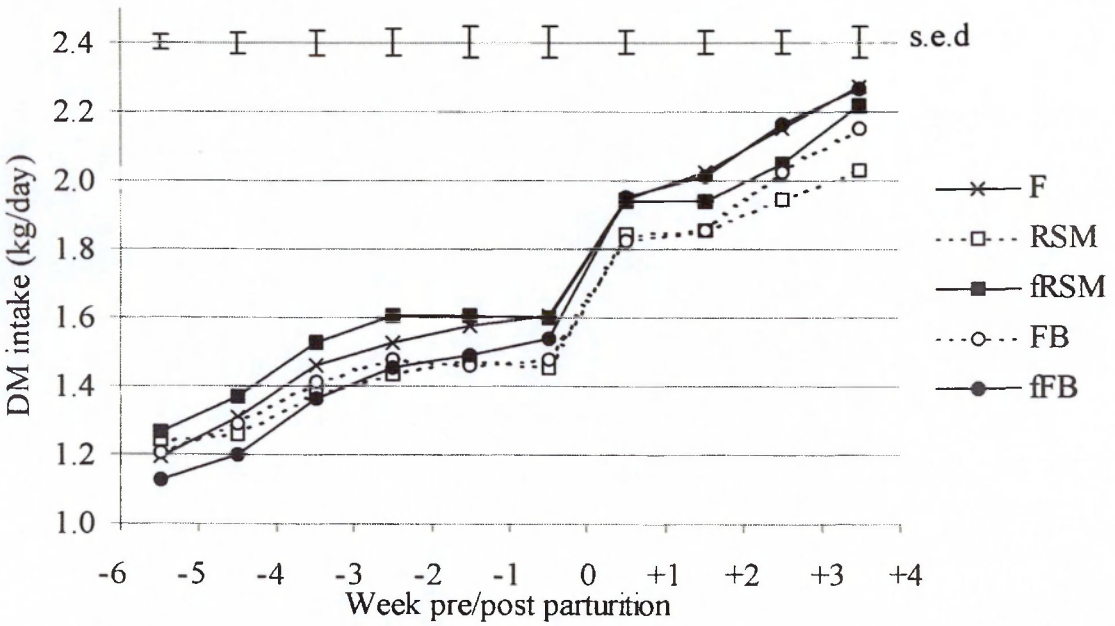

Figure 4.2 Total dry matter intake (DM; kg/day) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

Total calculated ME intake (MJ/ewe/day; Figure 4.3) increased on all treatments from 6 weeks *pre partum* (overall mean of 12.7 MJ ME/ewe/d) to parturition (17.0 MJ ME/ewe/day). After parturition, the ME intake by ewes increased on all diets, rising from 22.1 MJ ME/ewe/day during the first to 24.0 MJ ME/ewe/day during the fourth week of lactation. No significant effect of treatment on the ME intake by *pre partum* ewes was observed during any week of pregnancy. However, in the *post partum* period ewes fed diets containing formaldehyde treated protein sources had a significantly higher calculated ME intake than those fed untreated protein sources in all weeks of lactation ($P<0.05$). In addition, ewes fed concentrates containing fishmeal had higher ME intakes in the second and the third ($P<0.05$) week of lactation compared with the mean ME intake of ewes on other treatments, whilst ewes fed concentrates containing field beans had a higher ME intake compared to ewes fed diets containing rapeseed meal in the third ($P<0.05$) week of lactation. Whilst statistically significant differences in ME intake occurred during lactation, absolute differences were small.

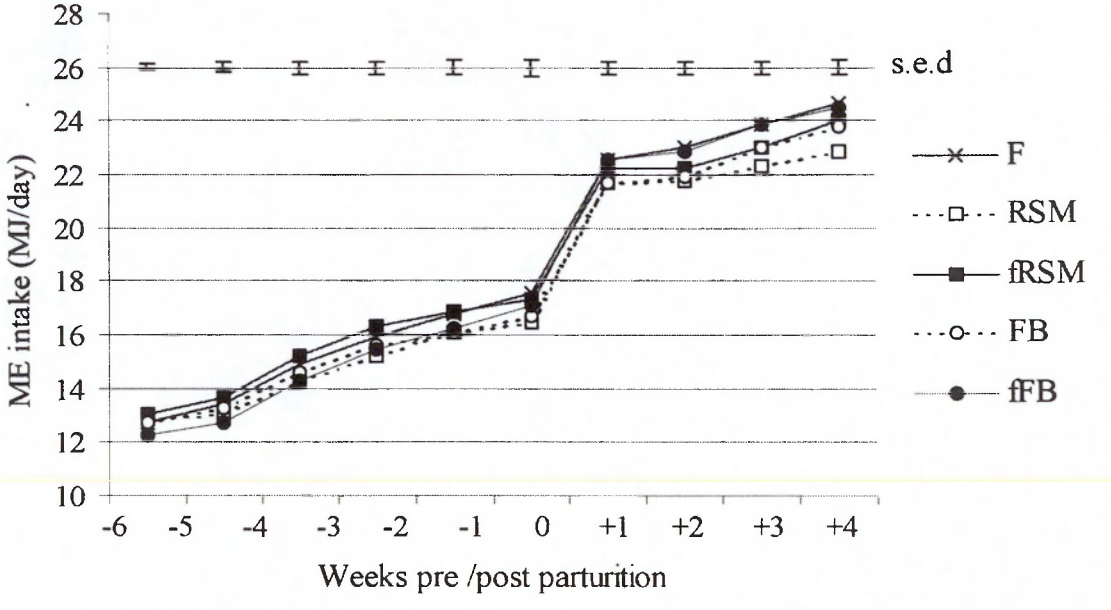

Figure 4.3 Calculated intake of metabolisable energy (ME; MJ/day) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

Total calculated DUP intake (g/ewe/day; Figure 4.4) increased on all treatments from 6 weeks *pre partum* (overall mean of 26 gDUP/ewe/day) to parturition (48 gDUP/ewe/day). After parturition the DUP intake increased in ewes fed all diets, rising from an overall mean intake of 75 gDUP/ewe/day in the first week of lactation to 81 gDUP/ewe/day during the third week and subsequently declining to 69 gDUP/ewe/day during the fourth week of lactation.

A lower calculated DUP intake was recorded in ewes fed diets containing fishmeal compared to ewes fed all other diets at 6, 5, 4, 3, 2 and 1 weeks *pre partum* ($P<0.05$) and during the first, second and third ($P<0.01$) week of lactation. Higher DUP intake was recorded for ewes fed diets containing formaldehyde treated protein sources compared to those fed untreated protein sources during each week of pregnancy and lactation ($P<0.001$). The DUP intake for ewes fed diets containing rapeseed meal was higher than those fed field beans at 6, 5, 4, 3, 2 and 1 weeks *pre partum* ($P<0.01$) and during the first week of lactation ($P<0.05$). A significant interaction also occurred, with formaldehyde treatment of field beans causing a bigger increase in the DUP intake than formaldehyde treatment of rapeseed meal ($P<0.05$).

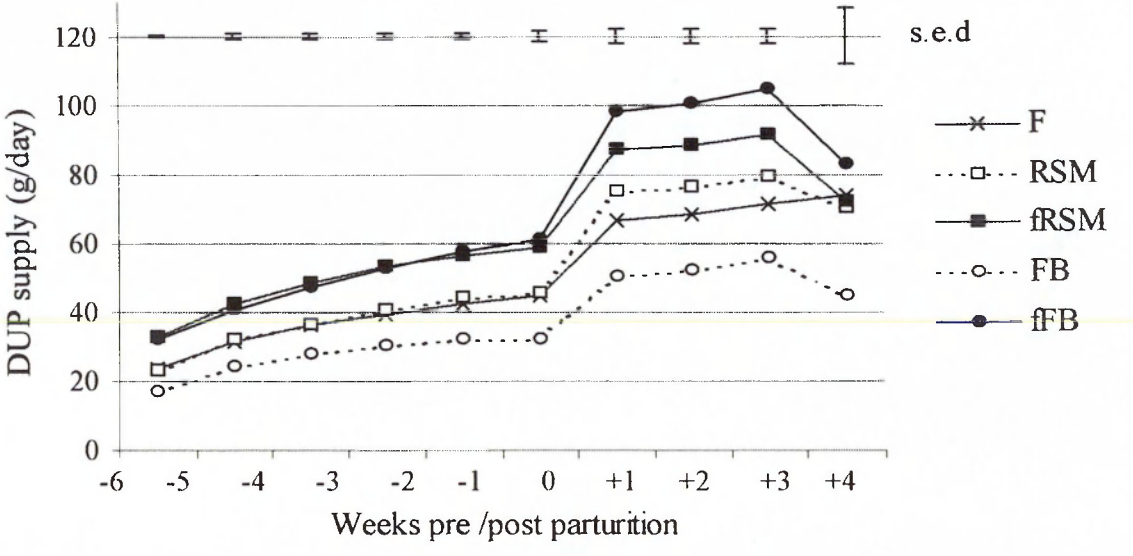

Figure 4.4 Calculated intake of digestible undegradable protein (DUP; g/day) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

Total calculated MP intake (g/ewe/day; Figure 4.5) increased on all treatments from 6 weeks *pre partum* (overall mean of 96 gMP/ewe/day) to parturition (144 gMP/ewe/day). After parturition the calculated MP intake increased from an overall mean intake of 205 gMP/ewe/day in the first week of lactation to 218 gMP/ewe/day during the fourth week.

Significant increases were calculated in the MP intake for ewes fed diets containing formaldehyde treated protein sources at during each week of pregnancy and during the first week of lactation ($P<0.001$). Significant increases were also calculated in the MP intake for ewes fed diets containing rapeseed meal at 6, 5, 4, 3 and 2 weeks *pre partum* ($P<0.05$). There was a significant interaction in *post partum* MP supply, with formaldehyde treatment of protein causing a bigger increase in the MP supply for ewes fed concentrates containing field beans than in ewes fed concentrates containing rapeseed meal in the first, second, third and fourth week of lactation ($P<0.05$).

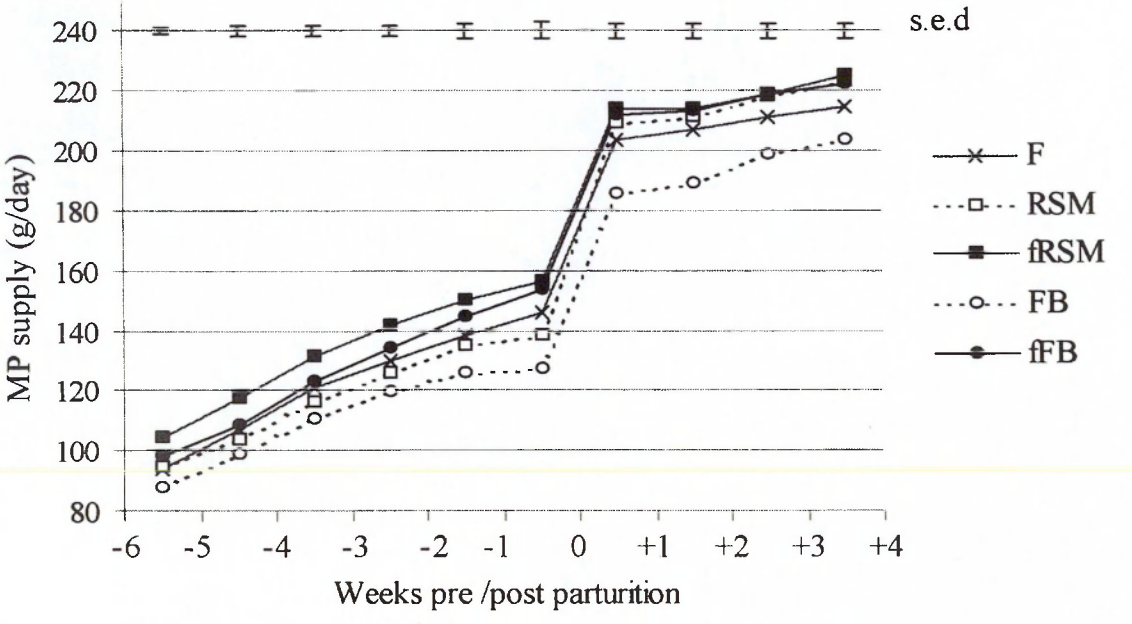

Figure 4.5 Calculated intake of metabolisable protein (MP; g/day) in ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

4.3.4 Ewe weight and condition score change

The effect of protein source and formaldehyde treatment on *pre partum* weight and condition score (CS) change is presented in Table 4.7. No effect of treatment on the ewe weight at 6 weeks *pre partum*, 1 week *pre partum* or on *pre partum* weight change was observed. However, in the *pre partum* period, ewes fed concentrate containing fishmeal (F) gained more condition than ewes on any of the other treatments (0.50 *v.* 0.23 respectively; $P<0.05$). There was no effect of treatment on the CS of ewes at 6 weeks *pre partum*, but at 1 week *pre partum* ewes fed diets containing rapeseed meal had a significantly higher CS than those fed field beans (3.24 *v.* 2.98; $P<0.05$). Ewes fed diets containing fishmeal were heavier at weeks 1 and 4 *post partum* ($P<0.05$) and tended to have a lower liveweight loss over this period compared to ewes on all other treatments ($P=0.055$). There was no effect of treatment on the CS of ewes at either 1 or 4 weeks *post partum*, but ewes fed diets containing rapeseed meal gained less condition than those fed field beans over this period ($P<0.05$).

Table 4.7 *Weight and condition score (CS) at 6 and 1 week pre partum, immediately post parturition and at 4 weeks post partum(kg), and pre partum (six to 1 week pre partum) and post partum (lambing to four weeks post lambing) weight (kg/week) and CS (units/week) change of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Pre partum weight:-										
At 6 weeks *pre partum*	72.0	73.8	73.4	72.5	73.2	1.79	NS	NS	NS	NS
At 1 week *pre partum*	83.8	84.4	84.0	84.1	81.6	2.10	NS	NS	NS	NS
Pre partum change	11.8	10.6	10.6	11.6	8.4	1.14	NS	NS	NS	NS
Pre partum CS:-										
At 6 weeks *pre partum*	2.83	3.01	2.92	2.83	2.86	0.125	NS	NS	NS	NS
At 1 week *pre partum*	3.33	3.23	3.25	3.09	2.87	0.163	NS	*	NS	NS
Pre partum change	0.50	0.22	0.33	0.35	0.03	0.159	*	NS	NS	NS
Post partum weight:-										
At 1 week *post partum*	72.7	70.1	70.4	69.3	68.2	1.77	*	NS	NS	NS
At 4 weeks *post partum*	70.9	66.3	67.7	64.5	64.5	2.32	*	NS	NS	NS
Post partum change	-1.8	-3.9	-2.6	-4.8	-3.8	1.28	NS	NS	NS	NS
Post partum CS:-										
At 1 week *post partum*	2.96	2.86	2.80	2.75	2.73	0.129	NS	NS	NS	NS
At 4 weeks *post partum*	3.08	2.87	2.84	3.02	2.99	0.155	NS	NS	NS	NS
Post partum change	0.13	0.01	0.08	0.27	0.26	0.130	NS	*	NS	NS

4.3.5 Colostrum production

4.3.5.1 Yield of Colostrum

Ewes fed concentrates containing field beans (FB and fFB) had a significantly lower initial yield of colostrum than those fed concentrates containing rapeseed meal (RSM and fRSM), with mean yields of colostrum from the ewes fed diets containing field beans and rapeseed meal of 793 and 492 g respectively ($P<0.05$). There was no effect of diet on secretion rate of colostrum between 12 - 16 hours *post partum* or on the calculated 24 hour yield (Table 4.8).

Table 4.8 *Initial yield of colostrum (g), subsequent secretion rates (12-16h; g/hour) and calculated 24 hour colostrum yield (g) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Initial yield	854	820	765	547	437	185.0	NS	*	NS	NS
Secretion rate	110	111	111	102	113	18.7	NS	NS	NS	NS
24 hour yield	3391	3369	3310	2886	3171	449.4	NS	NS	NS	NS

4.3.5.2 Colostrum composition and component yield at parturition

Ewes fed the concentrates containing fishmeal had a lower concentration of both DM and ash in their initial secretion of colostrum compared to ewes fed any of the other treatments ($P<0.05$; Table 4.9). There was no effect of fishmeal on any of the other constituents measured. Protein source and formaldehyde treatment had no effect on the concentration of DM, CP, fat, lactose, ash or IgG in colostrum at birth.

Ewes fed the concentrates containing field beans (FB and fFB) had lower yields of DM ($P<0.05$), CP ($P<0.05$), fat ($P<0.01$), lactose ($P<0.05$), ash ($P<0.05$) and IgG ($P<0.05$) compared with feeding diets containing rapeseed meal. No significant effect of formaldehyde

treatment of protein sources or of feeding the concentrate containing fishmeal was observed.

Table 4.9 *Initial concentration (g/kg) and yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Concentration (g/kg)										
DM	331	364	361	400	369	24.4	*	NS	NS	NS
CP	175	205	217	206	167	26.3	NS	NS	NS	NS
Fat	115	146	144	127	134	19.6	NS	NS	NS	NS
Lactose	12	16	14	12	16	4.2	NS	NS	NS	NS
Ash	10.9	11.8	11.4	12.9	11.8	0.66	*	NS	NS	NS
IgG	69	64	69	83	58	11.6	NS	NS	NS	NS
Yield (g)										
DM	263	288	270	193	171	54.8	NS	*	NS	NS
CP	148	182	166	107	85	37.5	NS	*	NS	NS
Fat	90	132	110	67	60	24.3	NS	**	NS	NS
Lactose	10.1	13.2	11.0	7.3	7.8	2.21	NS	*	NS	NS
Ash	8.7	9.3	8.6	6.3	5.6	1.80	NS	*	NS	NS
IgG	49.7	48.5	45.4	34.2	29.0	9.49	NS	*	NS	NS

4.3.5.3 Colostrum composition and component yield at 16 hours post partum

Concentrations of DM, CP, fat, lactose, ash and IgG in colostrum at 12-16 hours *post partum* were lower and concentration of lactose was higher than in the initial secretion of colostrum (Table 4.10). No main treatment effects on the concentration of constituents were observed. However, there were interactions for the concentrations of CP ($P<0.05$), ash ($P=0.050$) and IgG ($P<0.05$). Concentrations of these constituents were lower for ewes fed formaldehyde treated rapeseed meal than those fed untreated rapeseed meal, but were higher for ewes fed formaldehyde treated field beans than those fed untreated field beans.

No main treatment effects on the yield of colostrum constituents were observed. However, the yield of CP ($P<0.05$) and the yield of IgG ($P=0.091$) in colostrum was higher for ewes fed diets containing formaldehyde treated rapeseed meal compared to those fed untreated rapeseed meal, but to be lower for ewes fed concentrate containing formaldehyde treated field beans compared to those fed untreated field beans.

Table 4.10 *Concentration (g/kg) and yield (g/hour) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum secreted between 12 and 16 hours post partum from ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Concentration (g/kg)										
DM	223	249	238	233	260	24.7	NS	NS	NS	NS
CP	55	65	53	54	83	10.9	NS	NS	NS	*
Fat	92	93	100	87	90	13.2	NS	NS	NS	NS
Lactose	43	39	44	45	42	3.9	NS	NS	NS	NS
Ash	8.9	9.5	8.8	8.5	9.1	0.45	NS	NS	NS	NS
IgG	13	19	12	14	21	4.2	NS	NS	NS	*
Yield (g/h)										
DM	25.4	28.0	27.4	23.3	31.5	5.72	NS	NS	NS	NS
CP	6.4	8.0	6.0	5.2	9.9	2.17	NS	NS	NS	*
Fat	10.1	11.0	11.0	8.0	11.2	2.33	NS	NS	NS	NS
Lactose	4.7	4.3	4.9	4.6	4.7	0.54	NS	NS	NS	NS
Ash	1.0	1.1	1.0	0.8	1.1	0.20	NS	NS	NS	NS
IgG	1.7	2.4	1.3	1.6	2.4	0.74	NS	NS	NS	NS

4.3.5.4 Calculated yield of constituents over the first 24 hour post partum

Ewes fed concentrates containing field beans had lower calculated 24 hour yields of fat (g) compared to those fed concentrates containing rapeseed meal ($P<0.05$; Table 4.11). No other main effects of protein source or formaldehyde treatment were observed. However, there was a tendency for yield of DM ($P=0.097$), CP ($P=0.077$), ash ($P=0.090$) and IgG ($P=0.065$) in colostrum to be lower for ewes fed diets containing formaldehyde treated rapeseed meal compared to untreated rapeseed meal, but to be higher for ewes fed concentrate containing formaldehyde treated field beans compared to those fed untreated field beans.

Table 4.11 *Calculated yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum during the first 24 hours post partum for ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
DM	847	931	885	734	992	126.6	NS	NS	NS	NS
CP	296	367	304	227	312	57.4	NS	NS	NS	NS
Fat	321	384	362	250	318	58.3	NS	*	NS	NS
Lactose	118	112	124	113	115	8.8	NS	NS	NS	NS
Ash	32	35	31	25	33	4.6	NS	NS	NS	NS
IgG	88	103	76	67	88	18.1	NS	NS	NS	NS

4.3.6 Milk yield

4.3.6.1 Yield of milk, concentration of milk constituents and yield of milk constituents at 7 days post partum

There was no significant effect of protein type or of formaldehyde treatment of protein on the total secretion rate of ewes at 7 days *post partum* (Table 4.12). Similarly, there was no significant effect of protein type or of formaldehyde treatment on the concentration or yield of fat, protein, SNF or lactose in milk produced.

Table 4.12 *Secretion rate of milk (g), concentration of milk constituents(g/l) and secretion rate of milk constituents (g/hour) at 7 days post partum from ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	sed	Fish	Protein	Form	Int
Secretion rate of milk (g/h)										
	147	137	129	138	159	21.3	NS	NS	NS	NS
Concentration of milk constituents (g/l)										
Fat	101.3	90.8	98.0	97.0	90.5	10.33	NS	NS	NS	NS
Protein	35.6	35.6	35.1	34.8	35.0	0.46	NS	NS	NS	NS
SNF	90.9	90.7	88.6	89.5	91.1	1.52	NS	NS	NS	NS
Lactose	45.7	45.1	45.3	46.9	47.6	1.56	NS	NS	NS	NS
Secretion rate of milk constituents (g/h)										
Fat	15.5	11.9	12.8	13.2	15.0	2.35	NS	NS	NS	NS
Protein	5.2	4.9	4.7	4.8	5.6	0.76	NS	NS	NS	NS
SNF	13.3	12.7	12.0	12.4	14.4	2.01	NS	NS	NS	NS
Lactose	6.7	6.3	6.2	6.5	7.5	1.02	NS	NS	NS	NS

4.3.6.2 Yield of milk, concentration of milk constituents and yield of milk constituents at 21 days post partum

Ewes fed formaldehyde treated protein sources had a significantly lower milk yield than those fed untreated protein (103 *v.* 124 g/h for formaldehyde treated and untreated protein sources respectively; $P<0.05$; Table 4.13). There was no significant effect of either protein type or formaldehyde treatment on the fat, protein or lactose concentration in ewes milk taken at 21 days *post partum.* However, there was a tendency for ewes fed concentrates containing fishmeal (F) to produce milk with a higher concentration of SNF than ewes fed any of the other treatments (89.3 *v.* 87.7 g/l for ewes fed fishmeal and vegetable protein sources respectively; $P = 0.064$). The yield of fat tended to be lower ($P=0.050$), whilst the yield of protein, SNF, and lactose were significantly lower ($P<0.05$) when ewes were fed diets containing formaldehyde treated protein sources compared to those fed untreated sources (fRSM and fFB *v.* RSM and FB). In addition, ewes fed diets containing field beans tended to have a higher concentration of fat in their milk than those fed diets containing rapeseed meal ($P=0.050$). There was a tendency for an interaction, with ewes fed concentrates containing formaldehyde treated rapeseed meal having a higher lactose concentration in the milk than those fed diets containing untreated rapeseed meal (fRSM *v.* RSM), whilst ewes fed treated field beans had a lower lactose concentration compared to those fed untreated field beans (fFB *v.* FB; $P=0.072$).

Table 4.13 *Secretion rate of milk (g), concentration of milk constituents(g/l) and secretion rate of milk constituents (g/hour) at 21 days post partum from ewes which were fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	sed	Fish	Protein	Form	Int
Secretion rate of milk (g/h)										
	105	117	103	131	103	13.4	NS	NS	*	NS
Concentration of milk constituents (g/l)										
Fat	91.1	90.5	85.2	92.5	95.0	10.36	NS	NS	NS	NS
Protein	34.2	34.0	33.8	33.9	33.9	0.37	NS	NS	NS	NS
SNF	89.3	87.5	88.6	87.9	86.7	1.05	NS	NS	NS	NS
Lactose	49.1	48.1	49.0	48.4	47.3	0.73	NS	NS	NS	NS
Secretion rate of milk constituents (g/h)										
Fat	9.5	11.1	8.7	12.6	9.9	1.78	NS	NS	NS	NS
Protein	3.6	4.3	3.5	4.5	3.5	0.47	NS	NS	**	NS
SNF	9.4	11.0	9.0	11.6	8.9	1.16	NS	NS	**	NS
Lactose	5.2	6.0	5.0	6.3	4.9	0.61	NS	NS	**	NS

4.3.7 Litter birth weight and lamb growth rate

Lambs reared by ewes fed the concentrate containing fishmeal (F) had lower growth rates (246 *v.* 268 g/day respectively; $P<0.05$) and lower 28 day litter weights (21.9 *v.*23.6 kg respectively; $P<0.05$; Table 4.14) than ewes fed any of the other treatments. There was also a significant interaction ($P<0.05$), with the formaldehyde treatment of rapeseed meal increasing lamb growth rates, whilst formaldehyde treatment decreased growth rates in lambs from ewes fed concentrates containing field beans.

Table 4.14 *Litter birth weight (kg), 28 day weight (kg) and lamb growth rate (g/d) of lambs from ewes which were fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Litter birth weight	8.21	8.64	8.05	8.57	8.75	0.48	NS	NS	NS	NS
28 day litter weight	21.9	23.6	23.9	23.7	23.2	0.92	*	NS	NS	NS
Lamb growth rate	246	267	294	272	261	13.2	*	NS	NS	*

4.3.8 Ewe blood metabolites

Ewes fed the concentrate containing fishmeal (F) had a lower mean concentration of plasma NEFA in the *pre partum* period (0.47 *v.* 0.54 mmol/l respectively; $P<0.05$) and BHB (0.38 *v.* 0.51 mmol/l respectively; $P<0.05$) compared to ewes fed any of the other treatments. By contrast, after lambing, ewes fed the fishmeal diet (F) had significantly higher concentrations of plasma NEFA compared to ewes fed any of the other diets (0.64 *v.* 0.48 mmol/l; $P<0.01$; Table 4.15). Additionally, there was a significant interaction *pre partum* on the plasma NEFA concentration. Ewes fed formaldehyde treated rapeseed had lower plasma NEFA concentration than those fed untreated rapeseed meal, whilst ewes fed treated field beans had a higher

concentration than those fed untreated field beans ($P<0.05$). Plasma glucose concentration in the *pre partum* period was significantly higher in the ewes fed concentrates containing rapeseed meal (RSM and fRSM) than those fed field beans (FB and fFB; 2.61 *v.* 2.40 mmol/l; $P<0.01$). Concentrations were also higher in ewes fed concentrates containing formaldehyde treated protein sources (fRSM and fFB) than those fed untreated diets (RSM and FB; 2.60 *v* 2.41 mmol/l; $P<0.01$).

There were no main effects of either protein source or formaldehyde treatment on plasma concentrations of urea-N, albumin or total protein (Table 4.15). However, *post partum* urea-N tended to be higher ($P=0.077$) and albumin was significantly greater ($P<0.05$), in ewes fed formaldehyde treated rapeseed meal compared to those fed untreated rapeseed meal, but were lower when ewes were fed formaldehyde treated field beans compared to those fed untreated field beans.

Table 4.15 *Pre partum and post partum plasma concentrations of NEFA (mmol/l), BHB (mmol/l), glucose (mmol/l), urea-N (mmol/l), albumin (g/l) and total protein (g/l) of ewes fed concentrates containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation*

	Treatment means						Significance			
	F	RSM	fRSM	FB	fFB	s.e.d.	Fish	Protein	Form	Int
Mean pre partum:										
NEFA	0.47	0.59	0.52	0.50	0.55	0.039	*	NS	NS	*
BHB	0.38	0.47	0.47	0.55	0.54	0.056	*	NS	NS	NS
Glucose	2.52	2.48	2.73	2.33	2.47	0.096	NS	**	**	NS
Urea-N	7.0	6.6	6.9	6.6	6.8	0.44	NS	NS	NS	NS
Albumin	37.8	36.2	37.9	36.4	37.0	1.10	NS	NS	NS	NS
Total Protein	66.1	62.5	65.0	63.4	64.7	1.90	NS	NS	NS	NS
Mean post partum:										
NEFA	0.64	0.50	0.52	0.46	0.42	0.067	**	NS	NS	NS
BHB	0.66	0.65	0.75	0.77	0.69	0.116	NS	NS	NS	NS
Glucose	2.90	3.01	3.04	3.03	3.02	0.125	NS	NS	NS	NS
Urea-N	8.2	7.5	8.4	8.7	8.1	0.53	NS	NS	NS	NS
Albumin	39.7	38.7	41.2	40.0	37.8	1.41	NS	NS	NS	*
Total Protein	65.8	66.0	68.1	66.2	63.3	2.48	NS	NS	NS	NS

4.3.8.1 Ewe blood metabolic profiles

Plasma NEFA concentration increased in ewes fed all treatments from 4 weeks to 2 weeks *pre partum* and subsequently declined towards parturition (Figure 4.6). In lactation, plasma NEFA concentration increased from 0.43 mmol/l at 1 week to 0.69 mmol/l at 2 weeks of lactation and then subsequently declined to 0.42 mmol/l at 4 weeks.

In the *pre partum* period, ewes fed concentrates containing fishmeal (F) had a lower plasma NEFA concentrations at weeks 2 and 1 *pre partum* ($P<0.01$) and had higher concentrations compared to all other ewes at week 2 of lactation ($P<0.05$). Ewes fed formaldehyde treated protein sources tended to have a higher concentration of plasma NEFA than those fed untreated protein sources at 4 weeks *pre partum* ($P=0.083$) and at 1 week *post partum* ($P=0.052$). However, at 4 weeks *post partum* ewes fed formaldehyde treated protein sources had lower concentrations than those fed untreated protein sources ($P<0.05$). At 1 week *pre partum* ewes fed formaldehyde treated rapeseed had a lower plasma NEFA concentration than those fed untreated rapeseed meal, whilst ewes fed treated field beans had a higher concentration than those fed untreated field beans ($P<0.05$).

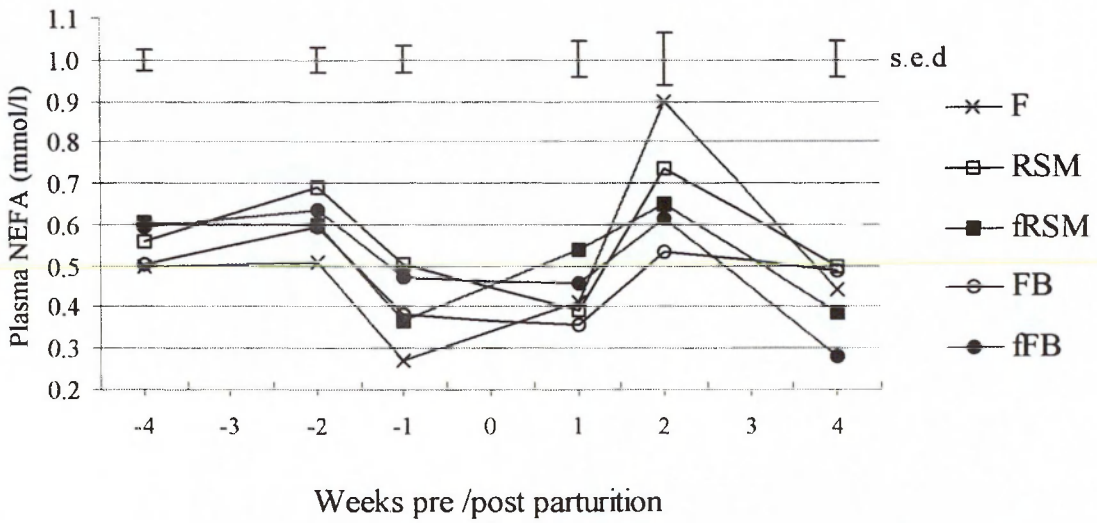

Figure 4.6 Concentrations of plasma NEFA (mmol/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

The weekly concentration of plasma BHB increased slightly on all treatments from week 4 to week 1 *pre partum* (Figure 4.7). In lactation, plasma BHB concentration increased from a mean value of 0.53 mmol/l at 1 week *post partum* to 1.01 mmol/l at 2 weeks *post partum* and then subsequently declined to 0.58 mmol/l at 4 weeks *post partum*.

Ewes fed the concentrate containing fishmeal had significantly lower plasma BHB concentrations compared to all other ewes at weeks 2 and 1 week *pre partum* ($P<0.01$), and tended to have lower plasma BHB concentration at week 1 ($P=0.094$) and week 4 ($P=0.052$) *post partum*. Ewes fed diets containing rapeseed meal tended to have lower plasma BHB concentrations than ewes fed diets containing field beans at 2 weeks *pre partum* ($P=0.051$). At 1 week *post partum*, formaldehyde treatment tended to decrease plasma BHB concentration in ewes fed rapeseed meal, but increased plasma BHB concentration in ewes fed field beans ($P=0.059$).

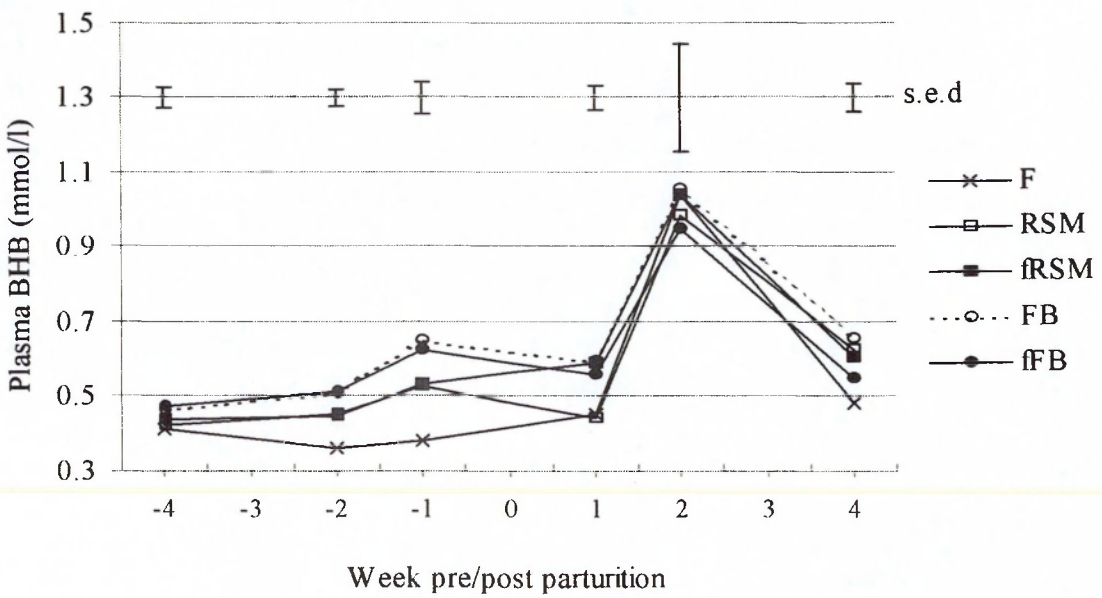

Figure 4.7 Concentrations of plasma BHB (mmol/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

Mean plasma glucose concentrations were higher during lactation than pregnancy (Figure 4.8). Ewes fed diets containing rapeseed meal had significantly higher plasma glucose concentrations compared to those fed field beans at weeks 2 (2.67 *v.* 2.46 mmol/l; $P<0.05$) and 1 (2.65 *v.* 2.43 mmol/l; $P<0.05$) *pre partum.* There was no effect of formaldehyde treatment on plasma glucose concentrations in either the *pre partum* or the *post partum* period.

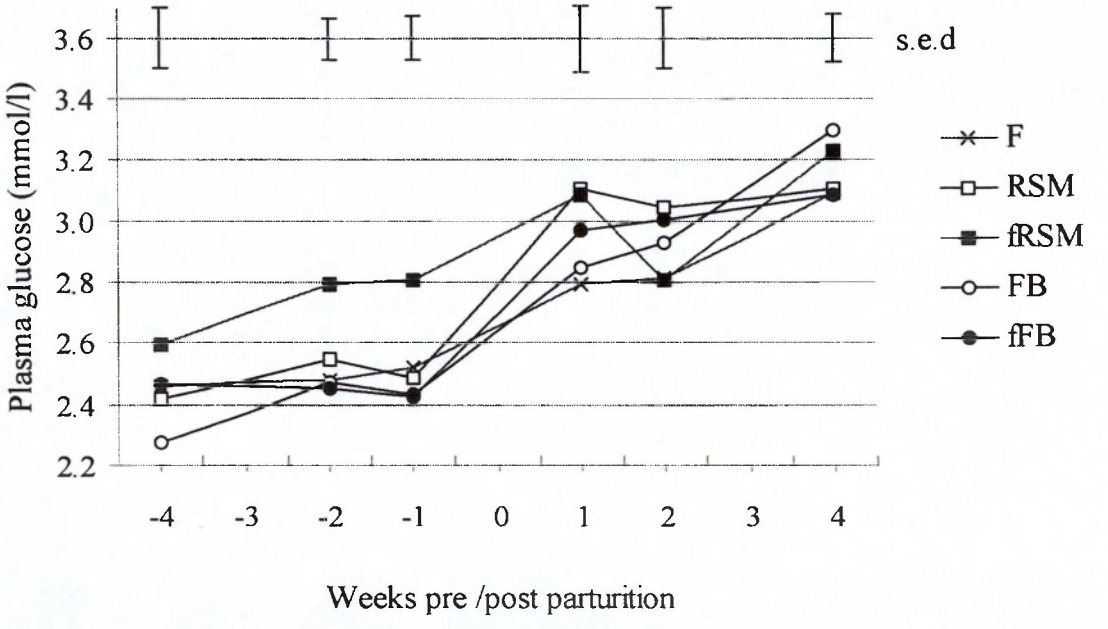

Figure 4.8 Concentrations of plasma glucose (mmol/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

The mean concentration of plasma urea-N decreased from week 4 to week 2 *pre partum* and subsequently increased towards parturition (Figure 4.9). In lactation, mean plasma urea-N concentration increased from 8.0 mmol/l at 1 week of lactation to 9.2 mmol/l at 2 weeks and then subsequently declined to 7.2 mmol/l at 4 weeks of lactation. There were no effect of feeding diets including fishmeal on the plasma urea-N concentration *pre partum* or *post partum*. A significant interaction occurred at 4 weeks *pre partum* and at 2 weeks *post partum*, with ewes fed formaldehyde treated rapeseed meal having higher plasma urea-N concentrations than ewes fed untreated rapeseed meal, but those fed formaldehyde treated field beans having lower concentrations than those fed untreated field beans ($P<0.05$).

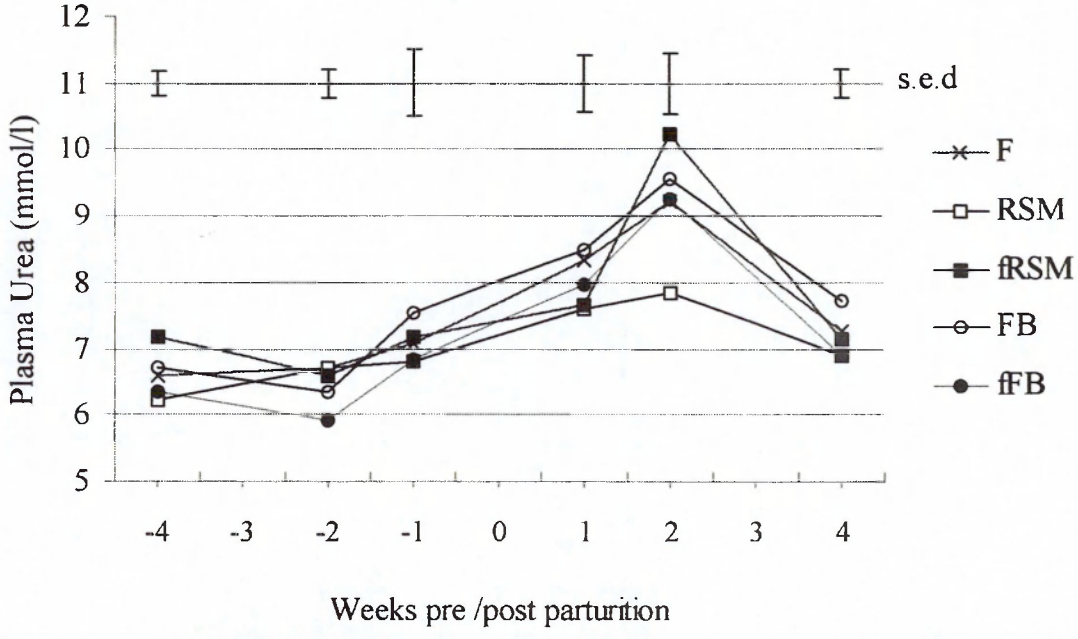

Figure 4.9 Concentrations of plasma urea-N (mmol/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

The concentrations of plasma albumin did not vary greatly over the period of the experiment (Figure 4.10). There was no effect of protein source or formaldehyde treatment on plasma albumin concentration during the *pre partum* period. However, in the fourth week of lactation, ewes fed diets containing rapeseed meal had a significantly higher plasma albumin concentrations than those fed diets containing field beans (38.2 *v.* 35.9 g/l respectively; $P<0.01$). A significant interaction occurred in the first week of lactation. Ewes fed formaldehyde treated rapeseed meal had higher concentrations of plasma albumin than those fed untreated rapeseed meal, whilst ewes fed formaldehyde treated field beans had lower concentrations than those fed untreated field beans ($P<0.05$).

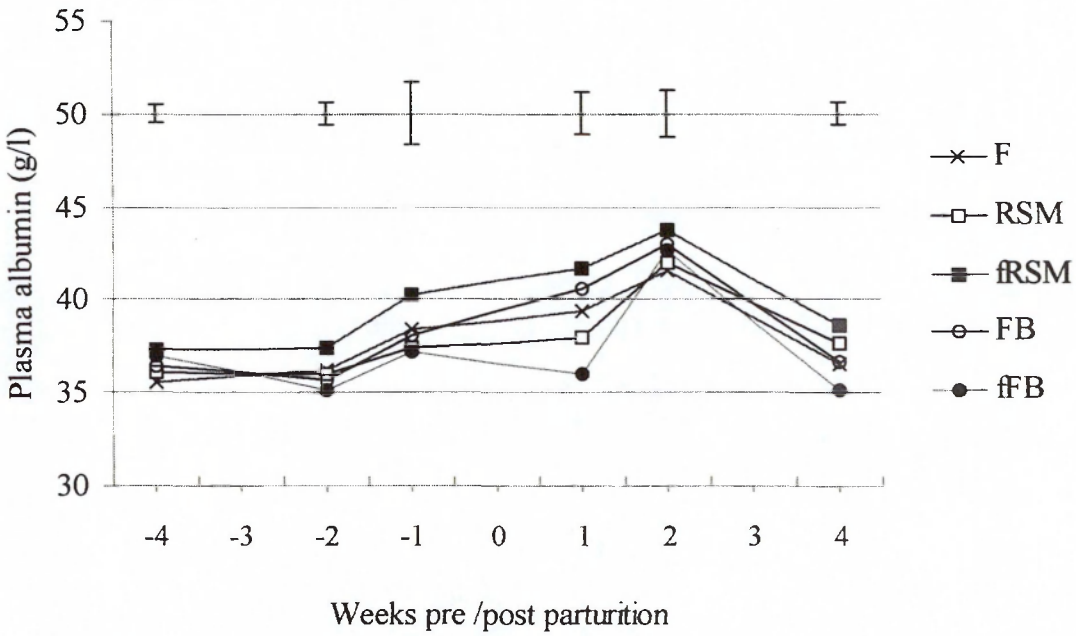

Figure 4.10 Concentrations of plasma albumin (g/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

The concentration of plasma total protein decreased on all treatments from weeks 4 to 2 *pre partum* and subsequently increased slightly towards parturition (Figure 4.11). In lactation, plasma total protein concentration increased from 64.5 g/l at 1 week of lactation to 70.3 g/l at 2 weeks and then subsequently declined to 62.9 g/l at 4 weeks of lactation. Ewes fed concentrates containing fishmeal had significantly higher plasma total protein compared to all other ewes at 2 weeks *pre partum* (64.7 *v.* 61.6 g/l respectively; $P<0.05$). There was no other effect of protein source or formaldehyde treatment on *pre partum* plasma total protein. In the *post partum* period, ewes fed diets containing rapeseed meal had higher plasma total protein concentrations compared to those fed field beans (65.0 *v.* 60.7 g/l; $P<0.05$).

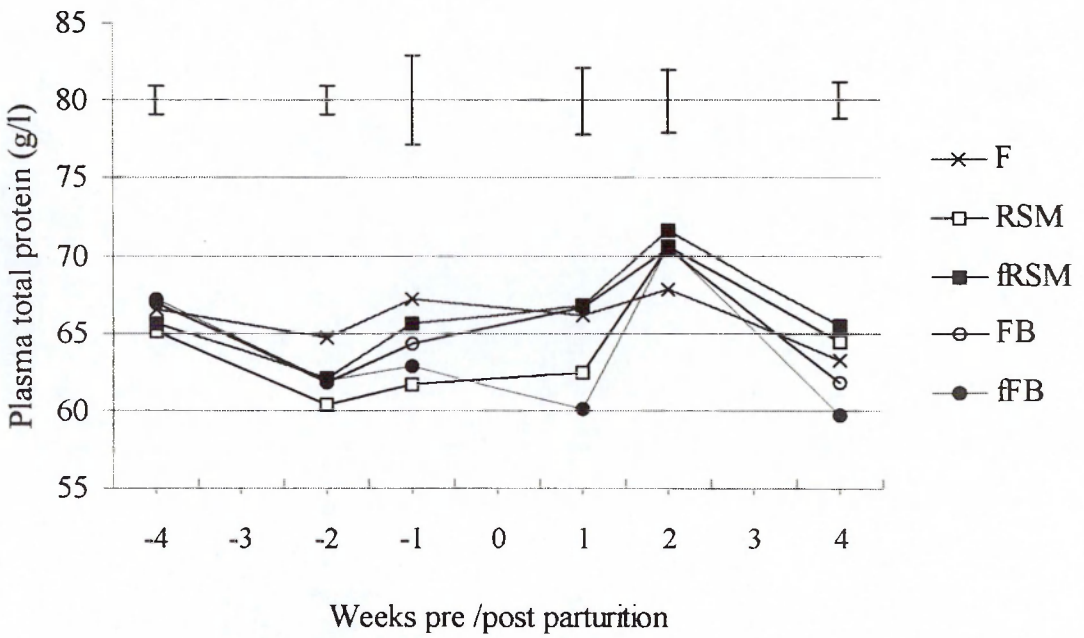

Figure 4.11 Concentrations of plasma total protein (g/l) of ewes fed concentrate containing fishmeal (F), rapeseed meal (RSM), formaldehyde treated rapeseed meal (fRSM), field beans (FB) or formaldehyde treated field beans (fFB) during late pregnancy and early lactation

4.4 DISCUSSION

4.4.1 Summary of main results

Ewes fed diets containing fishmeal had a higher *pre partum* condition score gain and lower concentrations of plasma BHB and NEFA ($P<0.05$) and produced colostrum at birth with a lower DM and ash concentration, than ewes fed all other diets. Ewes fed fishmeal also had lambs which grew slower and had a lower litter weight at 28 days, than ewes fed all other diets ($P<0.05$). In the *post partum* period, ewes fed diets containing fishmeal had lower plasma concentrations of NEFA, compared with ewes fed all other diets ($P<0.01$). Ewes fed diets containing rapeseed meal had a higher total colostrum yield at birth ($P<0.05$), and had higher yields of DM ($P<0.05$), CP ($P<0.05$), fat ($P<0.01$), ash ($P<0.05$) and IgG ($P<0.05$) in colostrum than ewes fed diets containing field beans. In addition, ewes fed rapeseed meal also had a higher *pre partum* plasma glucose concentration ($P<0.01$) and gained less condition score in the *post partum* period compared with those fed field beans ($P<0.05$). Ewes fed formaldehyde treated protein sources had a increased *pre partum* glucose concentration and had a reduced yield of total milk ($P<0.05$), protein ($P<0.01$), solids not fat ($P<0.05$) and lactose ($P<0.05$) at 21 days *post partum*, compared with ewes fed untreated protein sources.

4.4.2 Protein supply

Concentrate containing fishmeal (F) had a higher rate of nitrogen (N) degradation (c) than the formaldehyde treated protein sources, with a higher readily soluble N fraction (a) than any of the other concentrates. Treatment of rapeseed meal and field beans with formaldehyde reduced the soluble N fraction (a) and the rate of N degradation (c) of the potentially degradable N fraction (b), with the decrease in 'c' being greater in the concentrates containing field beans (FB and fFB) than in the concentrates containing rapeseed meal (RSM and fRSM). The reduction in N degradability of vegetable proteins by formaldehyde treatment observed in the

current experiment has been well documented by other authors (Varvikko *et al.*, 1983; Setala and Syrjala-Qvist, 1984; Antoniewicz *et al.*, 1992; Hadjipanayiotou, 1992; Subuh *et al.*, 1994; Hadjipanayiotou and Photiou, 1995; Rodehutscord *et al.*, 1999). In agreement with the current study, Witt *et al.* (1999a) found that the reduction in degradability of soya-bean meal treated with formaldehyde (Sopralin; Trouw Nutrition, UK) was caused by a reduction in both the readily soluble N fraction (*a*) and in the rate of N degradation (*c*) of the potentially degradable N (*b*) fraction.

The observed reductions in the soluble N fraction (*a*) and the rate of degradation (*c*) of the potentially degradable N fraction (*b*) as a result of formaldehyde treatment of the test protein increased the calculated MP supply both *pre partum* and *post partum*. Diets containing formaldehyde treated protein sources were formulated to increase the DUP supply without any concurrent decrease in microbial protein supply as the diets were formulated to have an supra-optimal ERDP:FME ratio. In addition, concentrates containing rapeseed meal also had a higher calculated MP supply than concentrates containing field beans. This was due to both the higher than formulated CP concentration and the lower rate of degradation (*c*) of the potentially degradable fraction (*b*) for diets containing rapeseed meal compared to those containing field beans. Fishmeal is widely regarded as a good source of undegradable protein (Gonzalez *et al.*, 1982). However, in the current experiment, the concentrate containing fishmeal (F) was relatively rumen degradable, with the whole diet supplying a lower calculated amount of DUP, both during pregnancy (36 g/d) and lactation (69 g/d) than the diet containing formaldehyde treated rapeseed meal (49 g/d in pregnancy and 89 g/d in lactation; fRSM) or formaldehyde treated field beans (48 g/d in pregnancy and 101 g/d in lactation; fFB). Although fishmeal is generally considered a good source of DUP, large variations in the degradability of different fishmeal samples have also been observed in other studies (Robinson, 1987). Ngongoni *et al.*

(1989) reported that the degradability coefficients obtained by the polyester bag technique indicated that the soya-bean meal and fishmeal proteins tested would supply similar amounts of undegraded protein.

4.4.3 Ewe and lamb performance

4.4.3.1 Straw intake

In late pregnancy, feed intake is often under physical (due to the reduction in the effective size of the rumen) rather than physiological control (Forbes, 1986) and other metabolic factors may regulate intake at this time (Ingvartsen and Anderson, 2000). It is therefore not surprising that, in the current experiment no effect of treatment on the *pre partum* intake of straw was observed. In lactation, however, there was a significant increase in straw intake due to formaldehyde treatment (fRSM and fFB *v.* RSM and FB; $P<0.05$) and a tendency for the concentrate containing fishmeal (F) to support higher mean levels of intake ($P=0.092$). Silva and Ørskov (1988) found that male sheep supplemented with fishmeal had an increased intake of barley straw compared to those receiving similar levels of CP from the more rumen degradable soya-bean meal. They concluded that the effect of fishmeal on intake may be mediated through an increase in the amino acid supply to the host animal rather than on rumen changes in degradation of fibre. Newbold (1994) stated that in situations where the overall MP:ME ratio in the diet is above the optimum, intake responses have been observed in dairy cows. Intake response is usually greatest when the ratio of MP:ME in the basal forage is less than optimum, thus increasing intake of such a forage would bring the ratio nearer the optimum (Newbold, 1994). The basal forage used in the current experiment was winter barley straw and would have a sub-optimal MP:ME ratio (AFRC, 1993), therefore the increase in straw intake observed (RSM and FB *v.* fRSM and fFB) may be in response to an increased MP supply due to formaldehyde treatment. If the intake response to MP is caused by an increase in the host

animal amino acid supply (Silva and Ørskov, 1988) then the extent of the response would be dependant on the most limiting essential amino acid within the MP (Newbold, 1994). Given that the amino acid supply from fishmeal is usually considered to be superior to that of vegetable proteins (Ngongoni *et al.* 1989) this may explain the high straw intake on the diet F, even though the calculated MP supply was not significantly increased.

4.4.3.2 Colostrum production

Initial colostrum yield, constituent concentration and constituent yield are all particularly variable between individual sheep (Hall and Egan, 1988; Pattinson *et al.*, 1995; O'Doherty and Crosby, 1997) and therefore it is not surprising that nutritional induced differences in colostrum yield have only been reported when there have been fairly severe degrees of under nutrition (Mellor and Murray, 1985a; Mellor and Murray, 1985b; Hall and Egan, 1988; O'Doherty and Crosby 1997). However, in the current experiment, initial yield of colostrum and the initial constituent yield of DM, fat, CP, ash and IgG were significantly lower in ewes fed concentrates containing field beans (FB and fFB) compared to those fed diets containing rapeseed meal (RSM and fRSM; $P<0.05$). There is no obvious reason for this difference. However, diets containing field beans (FB and fFB) supplied an extra 32 g/d of dietary starch (327 and 359 g/d for diets containing rapeseed meal and field beans respectively). High levels of dietary starch shift volatile fatty acid production in the rumen away from acetate and towards propionate (Reynolds *et al.*, 1997). Many studies have reported a decrease in milk fat concentration due to starch supplementation (e.g. Sutton, 1989). Such effects have been attributed directly to the reduction in lipogenic versus glucogenic precursors. Alternatively, the depression in milk fat concentration has been associated with increases in glucose turnover and plasma insulin concentrations (Evans *et al.*, 1975) causing a shift in energy balance towards body fat deposition and away from milk fat output. In contrast to the effects of dietary starch on milk

fat concentration, milk yield has been shown to be improved by feeding starch supplemented diets to dairy cows in early lactation (Reynolds *et al.*, 1996). In the current experiment, there was no similar effect of feeding concentrates containing field beans on 7 or 21 day milk yield. It is therefore unlikely that the lower colostrum present at birth is due to the higher starch concentrations in diets containing field beans.

It is also possible that the total amino acid supply, or the balance of individual amino acids in concentrates is important in determining initial colostrum yield. The increase in calculated *pre partum* MP supply seen in ewes fed diets containing rapeseed meal (RSM and fRSM) indicates an increase in total amino acid supply compared to ewes fed diets containing field beans (FB and fFB; Sloan, 1997) and may have resulted in an increase in the initial colostrum yield. However, increases in MP supply were also seen as a result of formaldehyde treatment, but no similar increase in colostrum yield was observed.

Robinson (1990a) stated that microbial protein is deficient in cystine for foetal growth in ewes during late pregnancy due to the high requirement of cystine for production of the lamb birth coat (Black, 1983). Interconversion of methionine to cystine may subsequently result in a deficiency of this amino acid as well (Robinson, 1990a). In addition to the requirements for methionine and cystine for the birth coat, there is a requirement for the rapidly growing foetus as well as for colostrum protein synthesis. It can be calculated from the dietary ingredients, that the combined concentration of methionine and cystine in concentrates containing field beans (FB and fFB) compared to those containing rapeseed meal (RSM and fRSM) was 5.10 and 1.10 g/kg fresh weight respectively. Therefore, although duodenal flow was not calculated, it may be that these amino acids were limiting the initial production of colostrum in concentrates containing field beans.

At 16 hours *post partum* mean concentrations of DM, CP, fat, ash and IgG in colostrum were lower and concentration of lactose higher compared with the initial colostrum. Similar observations have been made in Chapter 3 and by other authors (Mellor, 1990; Pattinson *et al.*, 1995) and would be expected as the gradual transition from colostrum to milk takes place. At 12-16 hours *post partum* a significant interaction occurred, with ewes fed formaldehyde treated field beans (fFB) producing colostrum containing a significantly higher protein and IgG concentration than those fed FB, whilst ewes fed formaldehyde treated rapeseed meal (fRSM) had lower protein and IgG concentration than ewes fed RSM ($P<0.05$). This contributed to a higher yield of CP ($P<0.05$) and a tendency for a higher IgG yield ($P=0.091$) in ewes fed fFB compared to FB and in RSM compared to fRSM. These differences would imply that there is either a reduction in the intestinal digestibility of rapeseed meal protein or of individual amino acids within the protein due to the formaldehyde treatment, reducing the amount absorbed or subsequent utilisation of the protein for colostrum synthesis. Alternatively, the amino acid balance of the rapeseed meal used may be inferior to that of field beans. Any production difference caused by the amino acid balance would be more apparent in the protected proteins as the ewe would be more reliant on the DUP portion of the protein. Some support for the former is given by the plasma urea-N concentrations, with a significant interaction occurring at 4 weeks *pre partum* and at 2 weeks *post partum* ($P<0.05$). Ewes fed formaldehyde treated rapeseed meal had a higher mean plasma urea-N concentration than ewes fed diets containing untreated rapeseed meal, whilst ewes fed formaldehyde treated field beans had lower concentrations than those fed diets containing untreated field beans. Plasma urea-N concentrations would be expected to be reduced by feeding diets which provide less rumen degradable protein. Concentrates containing formaldehyde treated rapeseed meal (fRSM) had a lower effective rumen N degradability than untreated rapeseed meal (RSM) and the fact that higher plasma urea-N concentrations were observed in fRSM compared to RSM implies that

it must be a post-ruminal effect.

4.4.3.3 Litter birth weight

Mean litter birth weight in this experiment was 8.44 kg which was similar to values reported in Chapter 3 and by other authors (Hill and Notman, 1998; Pattinson *et al.*, 1998 and Dawson *et al.*, 1999). From the data reviewed by Robinson and McDonald (1989), it may be reasonable to expect a litter birth weight response to switching to protein supplements which are more resistant to degradation in the rumen (RSM and FB *v.* FRSM and fFB). However, in the current experiment protein source and formaldehyde treatment failed to affect litter birth weight. The crucial difference between the current experiment and the stimulatory effect of rumen undegradable protein described by Robinson and McDonald (1989) is that in the current experiment, ME supply was formulated to meet the ewes requirements for maintenance and foetal growth, whilst the data reviewed by Robinson and McDonald (1989) was from ewes where ME intakes in late pregnancy were only adequate to meet the maintenance needs of the maternal body. In the current experiment, mean intakes of ME *pre partum* were 15.0 MJ/d of which 8.3 MJ/d was required for maternal maintenance and wool growth (AFRC, 1993). In situations described by Robinson and McDonald (1989), the lower ME intake (and hence FME intake) is likely to limit production of microbial protein and therefore switching to a more undegradable protein will allow MP production to approach the requirement of the ewe.

Data presented in Table 4.6 shows that the effect of formaldehyde treatment (fRSM and fFB *v.* RSM and FB) was to increase the MP supply to ewes during the *pre partum* period from 116 to 131 g/d. This difference in MP supply which formaldehyde treatment caused did not affect lamb birth weight. In agreement with the current work, Dawson *et al.* (1999) found no difference in litter birth weight when twin-bearing ewes were fed diets which supplied MP

ranging from 126 to 177 g/d. It also appears from the results of O'Doherty and Crosby (1998) that caution must be applied when using lamb birth weight as an indication of nutritional status. In the study of O'Doherty and Crosby (1998) ewes which were fed only 0.5 or 0.8 of their ME requirement had lambs within the expected weight range and they found no relationship between ME intake and lamb birth weight. In terms of protein nutrition, pregnant ruminants appear to be able to adapt their metabolism so that when dietary protein is limiting, foetal requirements can be met. Bell (1995) noted that maternal undernutrition had little effect on foetal uptake of amino acids in late-pregnant ewes, due to the active placental transport of most amino acids which is largely independent of changes in maternal blood concentration. In addition, increased hepatic protein synthesis (Bell, 1995) and decreased tissue protein stores (McNeill *et al.* 1994) have also been noted in late-pregnant ruminants fed low protein diets.

4.4.3.4 Ewe milk yield

No significant differences due to treatment were observed in the secretion rate, constituent concentration or in the yield of constituents of milk at 7 days *post partum*. At 21 days *post partum* ewes fed formaldehyde treated protein sources had a significantly lower milk yield than those fed untreated sources (fRSM and fFB *vs.* RSM and FB). Increased yields of milk may have been expected due to the improved MP supply as a result of formaldehyde treatment of protein sources. However, a review of the literature reveals that responses in milk yield to increased MP supply are variable. In agreement with the current experiment, Teweta *et al.* (1995) found no difference in the milk yield of lactating goats fed faba beans treated with 0.0, 4.3 or 5.4 g formaldehyde/kg. Similarly, Hadjipanayiotou (1992) found no increase in the yield of milk, fat or total solids in Damascus goats fed soya-bean meal treated with 1.2 g/kg of formaldehyde. In contrast to the current experiment, Hamilton *et al.* (1992) reported significantly higher yields of milk and protein in cows fed formaldehyde treated (5 g/kg)

sunflower meal compared to those fed untreated meal. Some of these differences observed may be due to the levels of formaldehyde or the protein source used. The lack of a response has sometimes been attributed to the over protection of protein sources leading to the reduced digestibility of the protein in the small intestine. However, the low levels used in the current experiment (3.80 and 2.38 g formaldehyde/kg for rapeseed meal and field beans respectively) in comparison with the experiments reported above are unlikely to lead to reduced intestinal digestibility. However, even with mild rates of formaldehyde treatment that have been shown to reduce rumen degradability, but not small intestine digestibility, responses in animal production have been negligible or very small (e.g. Kaim *et al.*, 1987). A possible reason for this is that even with low levels of formaldehyde treatment there is an irreversible reaction between formaldehyde and certain essential amino acids in the protein, rendering these amino acids unavailable for the animal (Ashes *et al.*, 1984; Erfle *et al.*, 1986). The disadvantages of this could outweigh the potential advantages of protein protection should these amino acids then become limiting in the concentrates containing treated proteins.

Work by Robinson *et al.* (1979) found increases in milk yield and lamb growth in ewes fed concentrates containing fishmeal, compared to those receiving the same intake of crude protein from soya-bean meal in weeks 2 to 3 of lactation but not, in weeks 4 and 5. It would also appear from the current results that any potential benefit from including high DUP protein sources in the diet of ewes would be reduced or even be detrimental when the ewes approach peak lactation. The reasons why formaldehyde treatment of field beans and rapeseed meal led to a lower milk yield at 21, but not at 7 days *post partum* may be a result of high rumen outflow rates at this stage of lactation. Mean outflow rates for ewes during the first and fourth week of lactation in the current experiment were calculated from AFRC (1993) to be 0.076 and 0.082 / hour respectively. At higher outflow rates the effective rumen N degradability of

proteins will be reduced (Ørskov *et al.*, 1983; AFRC, 1993). Although this will lead to an improvement in the DUP supplied by the concentrate, it appears that, in the current experiment, rumen efficiency was almost certainly compromised, with calculated ERDP:FME ratios of 10.8 and 8.7 for ewes during the fourth week of lactation fed untreated and formaldehyde treated concentrates respectively. In addition, any reductions in the amount of individual, available amino acids at the small intestine as a result of formaldehyde treatment would be more important at 21 days *post partum* than at 7 days *post partum*, as a greater proportion of the MP supplied to the small intestine is DUP.

4.4.3.3 Ewe weight and condition score change and lamb growth rate

When a lactating ruminant consumes a diet with an excess of MP relative to ME, providing the animal is in adequate body condition, it is possible that the increased supply of MP will induce increases in adipose mobilisation (Jones and Garnsworthy, 1988) or alternatively, the ruminant may increase the intake of a forage (with a low MP:ME ratio) which is provided *ad libitum*, and possible production responses could occur (Newbold, 1994).

When concentrations of plasma NEFA and BHB are considered together with condition score change, it appears that ewes fed concentrates containing fishmeal have a higher rate of adipose tissue deposition in the *pre partum* period, and have a higher rate of adipose tissue loss in the *post partum* period. This observation is supported by Sinclair *et al.* (1994) in lactating suckler cows, where feeding high DUP diets caused increased adipose mobilisation in cows that were in negative energy balance and increase adipose deposition in cows that were in positive energy balance. Ørskov *et al.* (1981) made similar observations in lactating dairy cows, where substituting fishmeal for groundnut meal reduced the calculated negative energy balance in cows fed a high level of intake, but increased the calculated negative energy balance for animals

on low levels of energy intake. No similar increase in the rate of adipose tissue mobilisation occurred in lactation with diets containing formaldehyde treated protein sources, suggesting that other factors besides the supply of DUP are important in the promotion of fat mobilisation and factors such as the amino acid balance within the DUP also have to be considered.

Ewes fed diets containing fishmeal produced lambs which grew significantly slower ($P<0.05$) and given that the main contributing factor to lamb liveweight gain is milk yield (Doney *et al.*,1981) it might be expected that ewes fed fishmeal may also have had a lower yield of colostrum and milk. However, there was no significant effect of feeding concentrates containing fishmeal on the initial yield of colostrum or secretion rates of colostrum at 12-16 hours *post partum* and milk at 7 or 21 days *post partum*. However, the mean yield of milk at 21 days was marginally lower for ewes fed diets containing fishmeal (105 g/h) compared to the mean yield of ewes fed all other treatments (113 g/h) and closer examination of lamb growth rates revealed that ewes fed diets containing fishmeal had lambs with comparable growth rates for the first 7 days of life but beyond that the growth rate was lower than for lambs reared by ewes on the four other treatments. Given these results, it is difficult to explain why diets containing fishmeal stimulated increases in body fat mobilisation during lactation (higher plasma NEFA concentrations) but failed to produce an increase in milk yield and produced lambs with significantly poorer growth rates. One potential problem with the use of plasma NEFA concentrations as an indication of rate of body fat mobilisation is that it takes no account of the rate of NEFA production or utilisation within the animal. Cowan *et al.* (1981) reported that lactating ewes that were mobilizing large amounts of body fat only had relatively low concentrations of plasma NEFA. The authors suggested that in such a situation the NEFA was being rapidly utilised.

4.5 CONCLUSIONS

Reductions in both the readily soluble N fraction and the rate of N degradation can be achieved by the treatment of vegetable protein sources with formaldehyde, but the extent of the reduction is dependent upon protein source. Formaldehyde treatment of vegetable protein sources can improve calculated MP supply to pregnant and lactating ewes. However, at 21 days of lactation, when outflow rates are high there appears to be a negative effect of formaldehyde treatment on ewe milk yield, which is possibly mediated through a reduction in rumen degradable protein supply. Inclusion of field beans in concentrates for pregnant ewes resulted in a significantly lower initial colostrum yield, which may have been a result of a poor amino acid supply from concentrates containing field beans. The fishmeal used in the current study had a higher rate of N degradation than expected and resulted in a low MP supply and significantly lower lamb growth rates.

CHAPTER 5

THE EFFECTS OF SOURCE AND FORMALDEHYDE TREATMENT OF DIETARY PROTEIN AND SUPPLEMENTATION WITH RUMEN PROTECTED METHIONINE ON THE METABOLISM AND PERFORMANCE OF HOUSED, STRAW FED, PREGNANT AND LACTATING EWES

5.1 INTRODUCTION

In Chapter 4, no significant benefit of formaldehyde treatment of protein sources was demonstrated. In addition, formaldehyde treatment significantly reduced the total yield of milk and constituent yield produced from ewes at 21 days *post partum*. A number of other studies have also shown that feeding formaldehyde treated vegetable protein sources has had no effect on performance in pregnant and lactating ruminants. For example, Tewatia *et al.* (1995) found that feeding lactating goats 400 g per day of faba beans that were treated with 4.3 or 5.4 g of formaldehyde per kg CP had no effect on total milk yield, milk fat or total solids and significantly decreased the total yield of milk protein compared to the feeding untreated faba beans. Similarly, Hadjipanayiotou, (1992) fed formaldehyde treated soya-bean meal and observed no change in the yield of milk, fat or total solids in lactating goats.

Methionine is considered to be the first limiting amino acid for milk protein synthesis in most production diets (Storm and Ørskov, 1984), and, due to interconversion from methionine, cystine can also become limiting in late pregnancy when the ewe has an additional requirement for cystine to develop the lamb birth coat (Robinson, 1990a). In addition, it is generally accepted that soya-bean meal contains a low concentration of methionine compared to other supplemental protein sources, such as fishmeal (Schingoethe, 1996).

Although formaldehyde treatment can increase the availability of total amino acids and total essential amino acids available to the ruminant (Barry, 1976), the relative proportions may change (Moshtaghi Nia and Ingalls, 1995; Mustafa *et al.*, 2000). Heat or formaldehyde treatment may reduce the quantity of some amino acids in the feed as well as reducing their subsequent digestion in the small intestine (Moshtaghi Nia and Ingalls, 1995). Rodehutscord *et al.* (1999) reported that rumen protected methionine increased both the body weight gain and clean wool growth in Merino wethers and attributed this to improvements in MP utilisation. The effect of rumen protected methionine on clean wool growth was approximately twice as high in sheep fed formaldehyde treated lupins compared to those fed untreated lupins (36 and 19% respectively), suggesting that treatment reduced the intestinal availability of this amino acid.

The current study was therefore designed to investigate the effects of including soya-bean meal, formaldehyde treated soya-bean meal or formaldehyde treated soya-bean meal with added rumen protected methionine in concentrates for housed, straw fed, pregnant and lactating ewes as an alternative to including fishmeal.

5.2 MATERIALS AND METHODS

5.2.1 Animals

At 103 days of gestation, 44 twin bearing Charollais x Lleyn (n=12), Charollais x Cambridge (n=4) Friesland x Lleyn (n=16) and Suffolk x North of England Mule (n=12) ewes were randomly allocated to one of four dietary treatments by breed, age, weight and condition score. All ewes were in lamb to Charollais rams.

5.2.2 Diets

The dietary treatments differed in the main protein source contained in the concentrate. These were fishmeal (F; 80 g/kg), soya-bean meal (S; 100 g/kg), formaldehyde treated soya-bean meal (TS; 100 g/kg: Sopralin™, Trouw Nutrition, UK) or formaldehyde treated soya-bean meal with added methionine (0.75 g/kg: Smartamine™ M, Rhône Poulenc, France; TSaa; 100 g/kg; Table 5.1). The calculated amount of crude protein supplied by the test proteins was designed to be similar (50 g/kg DM of crude protein), or approximately 25% of the total CP supplied by the concentrate. In addition, the inclusions rates of formaldehyde-treated and untreated soya-bean meal were the same. Formaldehyde treatment was carried out by applying 9.3 litres of a 30% formaldehyde solution to one tonne of soya-bean meal, which is equivalent to 2.8 g formaldehyde per kg of soya-bean meal. Concentrates were formulated to be isoenergetic (13.1 MJ ME/kgDM) and isonitrogenous (210 gCP/kg DM) and to have an ERDP:FME ratio of greater than 11.5 g/MJ during pregnancy. All concentrates were supplied on the same incremental scale (Table 5.2). The experiment ran from six weeks prior, to four weeks post lambing.

Table 5.1 *Dietary composition (g/kg) and predicted chemical composition (g/kgDM) of concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) fed to ewes during late pregnancy and early lactation*

	Treatment			
	Fishmeal (F)	Soya (S)	Sopralin (TS)	Sopralin + aa (TSaa)
Ingredient (g/kg)				
Barley	648	619	619	619
Sugar beet pellets (molassed)	100	100	100	100
Molasses	50	50	50	50
Field beans	61	62	62	62
Fishmeal	80			
Soya-bean meal		100		
Sopralin			100	
Sopralin / Smartamine				100
Urea	11	14	14	14
Megalac	20	25	25	25
Minerals / vitamins	30	30	30	30
ME (MJ/kgDM)	13.1	13.1	13.1	13.1
CP	210	210	210	210
EE	41	40	40	40
NDF	172	179	179	179
FME (MJ/kgDM)	11.7	11.7	11.7	11.7
ERDP[1]	139	161	146	145
ERDP[2]	132	150	135	133
DUP[1]	35	24	39	39
DUP[2]	41	34	50	50

[1] calculated at a rumen outflow rate (r) = 0.05

[2] calculated at a rumen outflow rate (r) = 0.08

Table 5.2 *Amount of concentrate (kg fresh weight/ewe/day) containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) fed to ewes during late pregnancy and early lactation*

Weeks *pre/post partum*	Concentrate
-6 to -5	0.6
-5 to -4	0.7
-4 to -3	0.8
-3 to -2	0.9
-2 to -1	1.0
-1 to 0	1.1
+0 to +4	1.6

5.2.3 Procedure and measurements

At 6 weeks *pre partum,* all ewes were individually penned and bedded on sawdust. Ewes were offered barley straw daily as a single feed at 0800 hours at proportionally 1.25 of the previous calculated intake. Straw refusals were removed and weighed (±10 g) at 0730 hours on Mondays, Wednesdays and Fridays. Straw offered was sampled weekly (Wednesday) by taking 6 equal samples of straw from six separate bales and a 200 g sample of refused straw was taken from individually penned sheep on Mondays. Concentrates were fed in 2 equal meals (0830 and 1630 hours) prior to parturition and in three equal meals (0800, 1300 and 1630 hours) thereafter. Concentrates were sampled weekly (Wednesday) by taking equal amounts (200 g) from four separate 25 kg bags. All samples of straw and concentrate were stored at 4°C in airtight containers until subsequent analysis.

Ewe liveweight, body condition score, litter birth weight, litter weekly weight, colostrum

production and milk production were measured and blood samples taken at 10 am during weeks 5, 3, 2 and 1 *pre partum* and at weeks 1, 2 and 4 of lactation by the methods described in Chapter 2.

Samples of colostrum were analysed for DM, fat, CP, IgG, lactose and ash, whilst samples of ewes milk were analysed for; total solids, fat, CP and lactose (Chapter 2). Blood plasma samples were analysed for total protein, albumin, urea-N, BHB, NEFA and glucose as described in Chapter 2.

5.2.4 The *in-situ* rumen degradability of nitrogen

The *in-situ* nitrogen degradability in the five concentrate feeds was measured using four rumen cannulated sheep.

5.2.4.1 Experimental animals, treatment and design

Four wether sheep aged 4 years with an average weight of 92 kg (s.d. 3.4 kg) and fitted with permanent rumen cannulae of 39 mm internal diameter, were assigned to a 4 x 4 latin square design and housed in individual, slatted floor pens, with free access to water and mineral licks. Animals were introduced to their surroundings and the trial diet two weeks prior to the insertion of polysynthetic fibre bags.

A basal concentrate diet was formulated to be a mean of the diets used in the production trial (Table 5.3).

Table 5.3 *Dietary composition (g/kg) of the basal concentrate fed to the rumen-cannulated wethers*

	Composition (g/kg)
Ingredient	
Barley	645
Sugar beet pellets (molassed)	103
Molasses	51
Fishmeal	21
Field beans	64
Soya-bean meal	26
Sopralin	26
Sopralin / Smartamine	26
Urea	14
Megalac	24

The wethers were then offered barley straw to achieve a diet with a ratio of barley straw:concentrate of 0.50:1, which was the same as the ratio consumed by the ewes in the production experiment. Concentrates were offered as a coarse mix in two equal feeds at 0830 and 1630 hours and straw at 0835 and 1635 hours. Diets were fed at 1.1 x maintenance.

Samples were collected and processed as described in Chapter 2.

5.2.5 Statistical analysis

The data was analysed using a completely randomised block design and subjected to analysis of variance (ANOVA) using Genstat 5 release 3.2 (Lawes Agricultural Trust, 1995). Treatment differences in litter birth weight and lamb growth rates were analysed using number of males in the litter as a co-variate. Lamb growth rate was estimated by linear regression.

5.3 RESULTS

The data from three ewes was excluded from the results. Of these, two ewes (one fed diet TS and one fed TSaa) aborted twin lambs prior to the expected lambing date, and one ewe (fed diet F) failed to eat her full allocation of concentrate on all days. All data collected from one other ewe during lactation (except colostrum data) was excluded from the results (fed diet TS) because she rejected one of her lambs.

5.3.1 Diet composition

The determined chemical composition of the concentrates and straw are shown in Table 5.4. The chemical composition of all four concentrates was similar and close to that predicted, with mean values for DM of 859 g/kg, CP 214 g/kg, EE 47 g/kgDM, ash 80 g/kgDM, NDF 152 g/kgDM, ADF 71 g/kg and ADIN 1.24 g/kgDM. The chemical composition of the fresh straw and the refusals were both very similar.

Table 5.4 *Determined chemical composition (g/kgDM) of concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (Tsaa), and determined chemical composition (g/kgDM) straw offered to and refused by ewes during late pregnancy and early lactation*

	F	S	TS	TSaa	Offered straw	Refused straw
g/kgDM						
DM (g/kg)	858	861	860	857	924	895
CP	206	217	218	216	30	32
EE	45	47	48	48	9	7
Ash	82	83	79	74	51	55
NDF	163	147	153	143	849	820
ADF	60	71	75	79	505	543
ADIN	1.02	1.43	1.34	1.16	1.41	1.52

5.3.2 Nitrogen degradability of the concentrates

N degradability coefficients of the concentrates are presented in Table 5.5. The concentrate containing fishmeal (F) had a high rate of N degradation (*c*), of the potentially degradable (*b*) fraction, and a high readily soluble (*a*) fraction. Formaldehyde treatment of soya-bean meal resulted in a lower soluble N fraction (*a*; 0.47 *v.* 0.42 for diets S and TS respectively) and in a lower rate of N degradation (*c*; 0.131 *v.* 0.108 for diets S and TS respectively) but did not significantly alter the potentially degradable N fraction (*b*; 0.50 and 0.49 for diets S and TS respectively). Diets TS and TSaa had a similar readily soluble N fraction (*a*; 0.42 and 0.43 respectively) and a similar rate of N degradation (*c*; 0.108 and 0.103 respectively), and a similar potentially degradable N fraction (*b*; 0.49 and 0.49 respectively). Formaldehyde treatment reduced the calculated effective N degradability at a ruminal outflow of 0.05 from 0.84 to 0.76 for diets S and TS respectively, whilst concentrate containing fishmeal (F) had an intermediate value of 0.77.

Table 5.5 *Nitrogen degradability coefficients for concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) fed to ewes during late pregnancy and early lactation*

	F	S	TS	TSaa	s.e.d.
a	0.51	0.47	0.42	0.43	0.009
b	0.35	0.50	0.49	0.49	0.012
c	0.151	0.131	0.108	0.103	0.014
a+b	0.86	0.97	0.91	0.92	0.013
r^2	95.7	98.4	96.1	95.8	
Effective N degradability (P):-					
r=0.05	0.77	0.84	0.76	0.76	0.010
r=0.08	0.74	0.79	0.70	0.70	0.011

Where *a* is the immediately soluble fraction, *b* is the insoluble but potentially degradable fraction, *c* is the constant rate of degradation of *b* and r is the rumen outflow rate per hour. Effective N degradability (P) was calculated according to the equations given in Chapter 2.

5.3.3 Feed and nutrient intake

The effect of diet on the intake of concentrate, straw, DM, ME and MP in the *pre partum* and *post partum* period is presented in Table 5.6. Weekly intake of straw, DM, ME, DUP and MP is presented in Figures 5.1, 5.2, 5.3, 5.4 and 5.5 respectively.

In the *pre partum* period, ewes fed concentrate containing fishmeal (F) and formaldehyde treated soya-bean meal with added amino acids (TSaa) had a significantly higher intake of straw DM, total DM and total ME than ewes fed diets containing soya-bean (S) and treated soya-bean (TS; $P<0.01$). Ewes fed diets F, TS and TSaa had a significantly higher calculated supply of *pre partum* DUP and MP than ewes on diet S ($P<0.001$). Ewes fed diet TSaa also had a higher *pre partum* DUP supply than ewes fed F and TS ($P<0.01$), but the actual difference in DUP supply was only small; 33, 33 and 36 g/d for diets F, TS and TSaa respectively. Ewes fed TSaa also had a significantly higher *pre partum* calculated MP supply than ewes fed diet TS (106 and 97 g/d respectively; $P<0.01$).

No significant effect of dietary treatment on ME intake was observed in the *post partum* period. However, ewes fed diets F and TSaa tended to have higher *post partum* intakes of straw DM ($P=0.063$) and total DM ($P=0.063$) than ewes fed diets S and TS. Ewes fed diet F, TS and TSaa had a significantly higher calculated supply of *post partum* DUP and MP than ewes on diet S ($P<0.001$). Ewes fed diet TSaa and TS also had a higher *post partum* DUP supply than ewes fed F ($P<0.001$).

Table 5.6 *Intake of concentrate (kgDM/d), straw (kgDM/d), total dry matter (DM; kg/d) and calculated intake of metabolisable energy (ME; MJ/d), digestible undegradable protein (DUP;g/d) and metabolisable protein (MP; g/d) in ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Pre partum:-						
Concentrate	0.73	0.73	0.73	0.73	-	-
Straw	0.53	0.40	0.39	0.53	0.052	**
DM	1.26	1.13	1.12	1.26	0.052	**
ME	12.9	12.1	12.0	12.9	0.33	**
DUP	33	22	33	36	1.0	***
MP	103	87	97	106	3.0	***
Post partum:-						
Concentrate	1.37	1.37	1.37	1.37	-	-
Straw	0.68	0.47	0.57	0.70	0.092	NS
DM	2.05	1.84	1.94	2.07	0.092	NS
ME	22.1	20.9	21.6	22.0	0.70	NS
DUP	74	61	84	88	1.8	***
MP	199	188	211	215	3.8	***

5.3.3.1 Intake of concentrate, straw, dry matter, metabolisable energy and metabolisable protein

The intake of straw increased in ewes on all treatments from week 6 (overall mean of 0.38 kg DM/ewe/day) to week 5 (0.48 kg DM/ewe/day) *pre partum* and remained relatively constant until 2 weeks *pre partum* (0.49 kg DM/ewe/day; Figure 5.1). The mean intake of straw was then reduced slightly in all treatments as parturition approached (0.45 kg DM/ewe/day during the final week before lambing; Figure 5.1). Straw intakes then increased up to the third week of lactation but declined in the fourth, with ewes eating 0.71 and 0.66 kg DM/ewe/day on average during the third and fourth week of lactation respectively. No significant effect of treatment on the straw DM intake of ewes was observed at 6 or 5 weeks pre-lambing, nor during the second, third or fourth week of lactation. However, from 4 weeks *pre partum* to 1 week *post partum* ewes fed diets S and TS had a significantly lower straw intake than those fed diets F or TSaa ($P<0.05$).

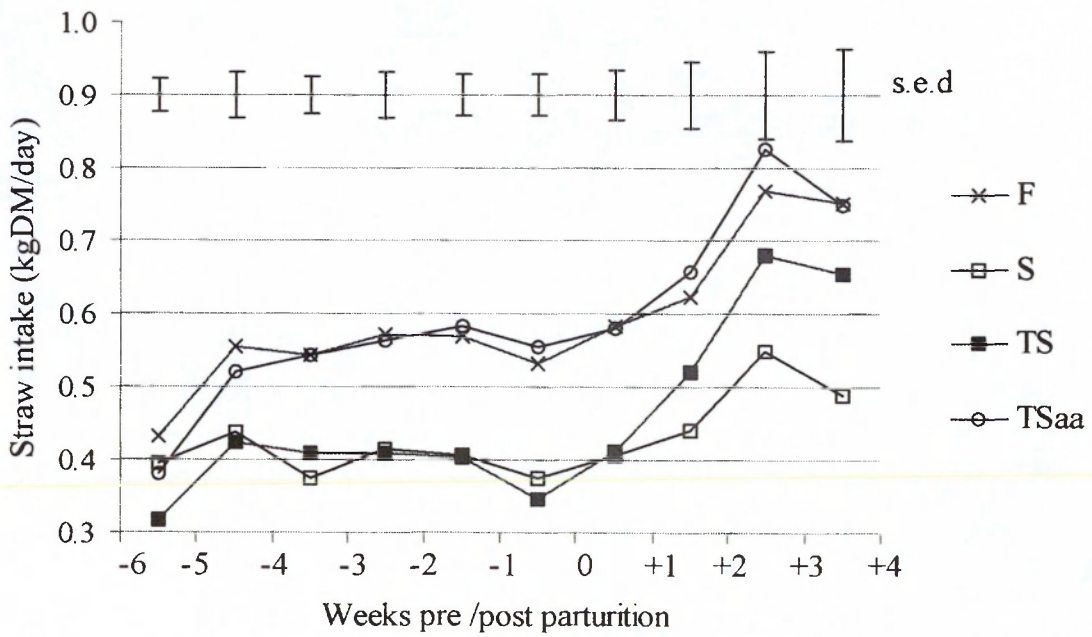

Figure 5.1 Intake of straw (kg DM/d) in ewes fed concentrate containing (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

Total DM intake (kg/ewe/d; Figure 5.2) increased on all treatments from 6 weeks *pre partum* to lambing. After parturition, DM intake was higher on all diets and in all weeks, compared to intakes *pre partum*. Ewe DM intake increased in ewes fed all diets as lactation progressed and reached a maximum in the third week of lactation (2.08 kgDM/d) and then subsequently declined slightly during the fourth week of lactation (2.03 kgDM/d).

No significant effect of treatment on the DM intake of ewes was observed at 6 or 5 weeks *pre partum*, nor during the second, third or fourth week of lactation. However, from 4 weeks *pre partum* to 1 week *post partum* ewes fed diets S and TS had significantly lower DM intake than those fed either F or TSaa ($P<0.05$).

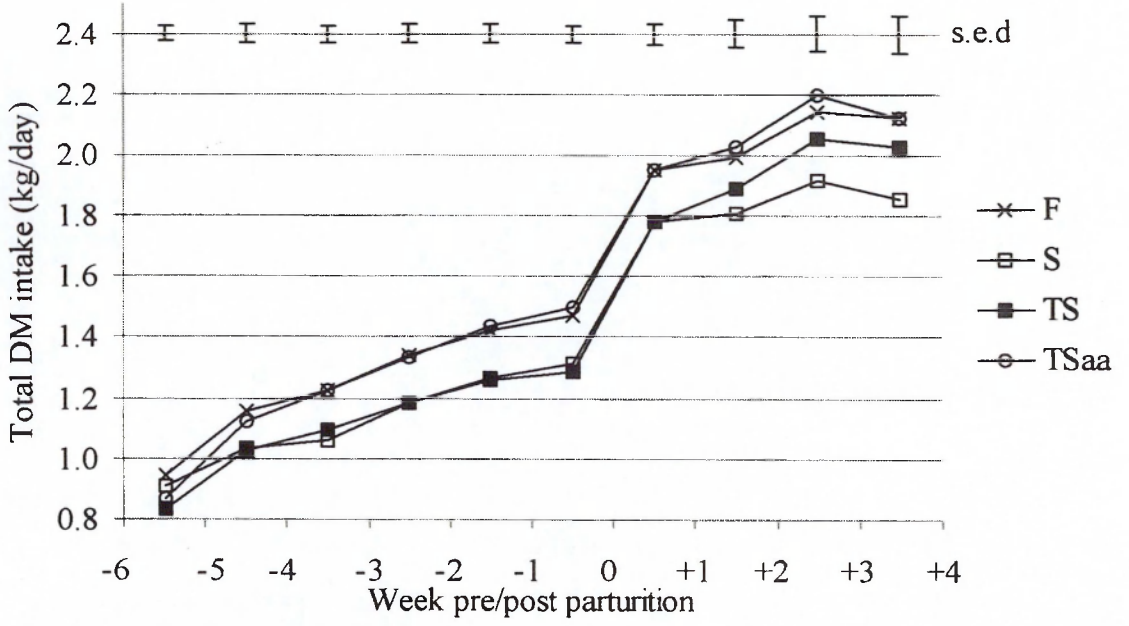

Figure 5.2 Total dry matter intake (DM; kg/d) in ewes fed concentrate containing (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) and formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

Total calculated ME intake (MJ ME/ewe/d; Figure 5.3) increased on all treatments from 6 weeks *pre partum* (overall mean of 9.2 MJ ME/ewe/d) to parturition (15.2 MJ ME/ewe/d). After parturition the calculated ME intake of ewes was higher on all diets, in all weeks, compared to ewes in the *pre partum* period. ME intake increased in ewes fed all diets as lactation proceeded, rising from 21.1 MJ ME/ewe/d during the first to 22.2 MJ ME/ewe/d during the fourth week of lactation. No significant effect of treatment on the ME intake of ewes was observed at 6 or 5 weeks *pre partum*, nor during the second, third or fourth week of lactation. However, from 4 weeks *pre partum* to 1 week *post partum* ewes fed diets S and TS had significantly lower ME intake than those fed F or TSaa ($P<0.05$).

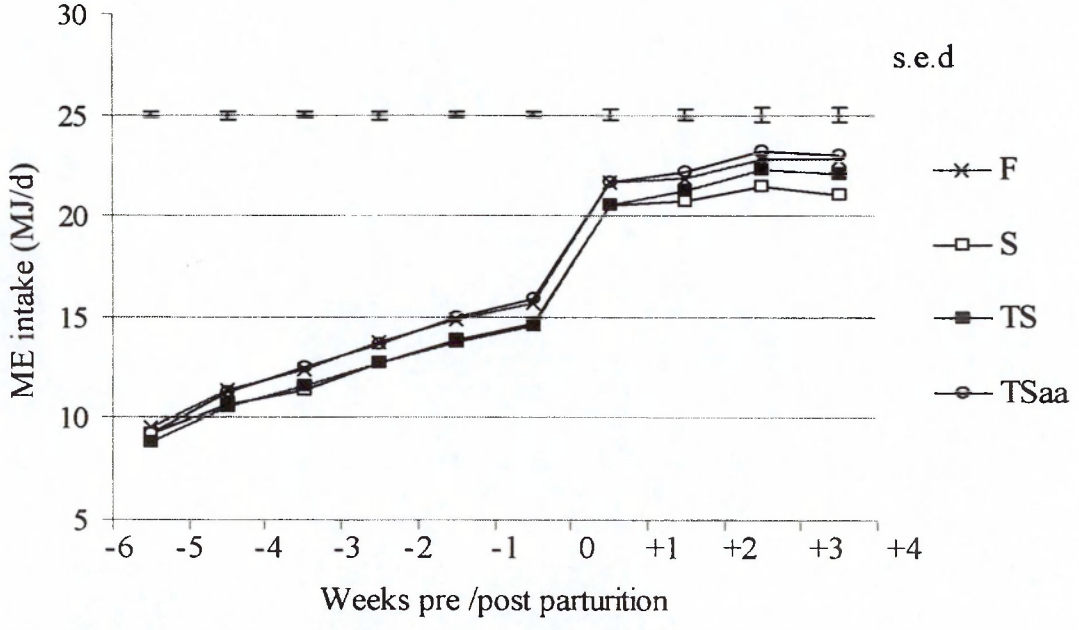

Figure 5.3 Calculated intake of metabolisable energy (ME; MJ/d) in ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

Total calculated DUP intake (kg/ewe/d) increased on all treatments from 6 weeks *pre partum* (overall mean of 13.7 g /ewe/d) to parturition (42.7 g/ewe/d; Figure 5.4). After parturition, the calculated DUP intake of ewes was higher on all diets, in all weeks, compared to ewes in the *pre partum* period and the calculated intake increased in ewes fed all diets as lactation proceeded, rising steadily from 73.4 g/ewe/d during the first to 78.6 g/ewe/d during the fourth week of lactation.

The calculated DUP intake of ewes fed diet S was significantly lower during each week of pregnancy and lactation than ewes fed diets F, TS or TSaa ($P<0.05$). At 6 weeks *pre partum*, ewes fed diet F had a significantly higher intake of DUP than those fed diets TS or TSaa ($P<0.05$), whilst from 4 weeks *pre partum* to lambing ewes fed diets F or TS had a significantly lower intake than those fed TSaa ($P<0.05$). During the first two weeks of lactation, ewes fed F or TS had a lower DUP intake than those fed TSaa and ewes fed F also had a lower intake than ewes fed diet TS ($P<0.05$), whilst during the third and fourth week of lactation ewes fed diet F had a lower DUP intake than ewes fed either diet TS or TSaa ($P<0.05$).

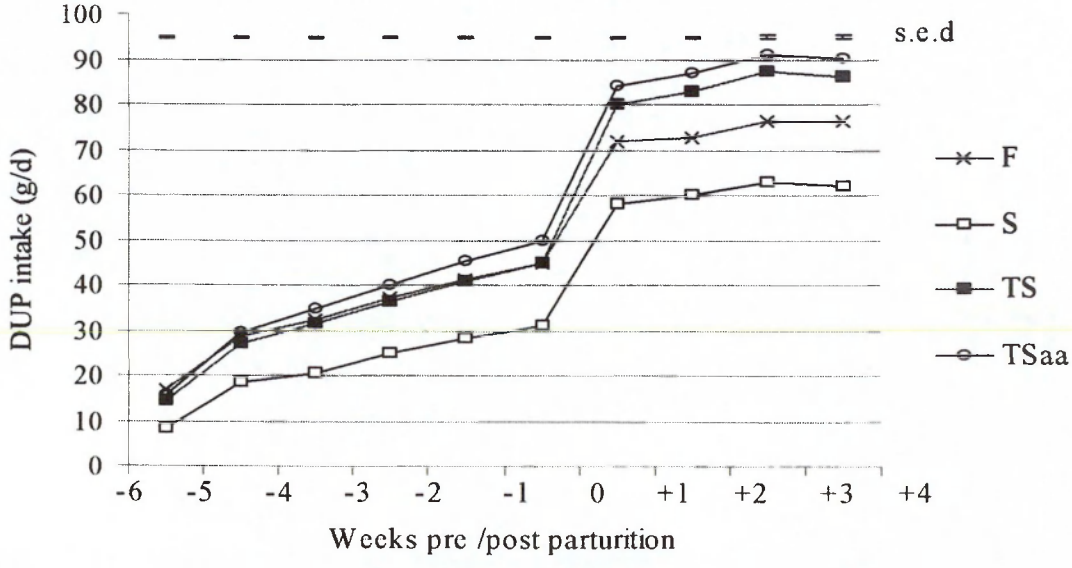

Figure 5.4 Calculated intake of digestible undegradable protein (DUP; g/d) in ewes fed concentrate containing (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

Total calculated MP intake (g/ewe/d) increased on all treatments from 6 weeks *pre partum* (overall mean of 61 gMP/ewe/d) to parturition (127 gMP/ewe/d; Figure 5.5). After parturition the calculated MP intake by ewes was higher on all diets, in all weeks, compared to ewes in the *pre partum* period and the calculated MP intake increased in ewes fed all diets as lactation proceeded, rising from an overall mean intake of 198 gMP/ewe/d in the first week of lactation to 204 gMP/ewe/d during the fourth week. The calculated MP intake of ewes fed diet S was significantly lower during each week of pregnancy and lactation than ewes fed diets F, TS or TSaa ($P<0.05$). At 6 weeks *pre partum*, ewes fed diet TS had a significantly lower intake of MP than those fed diet F ($P<0.05$), whilst from 4 weeks *pre partum* to lambing ewes fed diet TS had a significantly lower intake than those fed TSaa ($P<0.05$). During the final week of pregnancy, ewes fed diets F or TSaa had a higher MP intake than those fed TS ($P<0.05$). However, during the first week of lactation, ewes fed diets F or TS had a lower MP intake than those fed diet TSaa, whilst during the second, third and fourth week of lactation, ewes fed diets TS or TSaa had a significantly higher MP intake than ewes fed diet F ($P<0.05$).

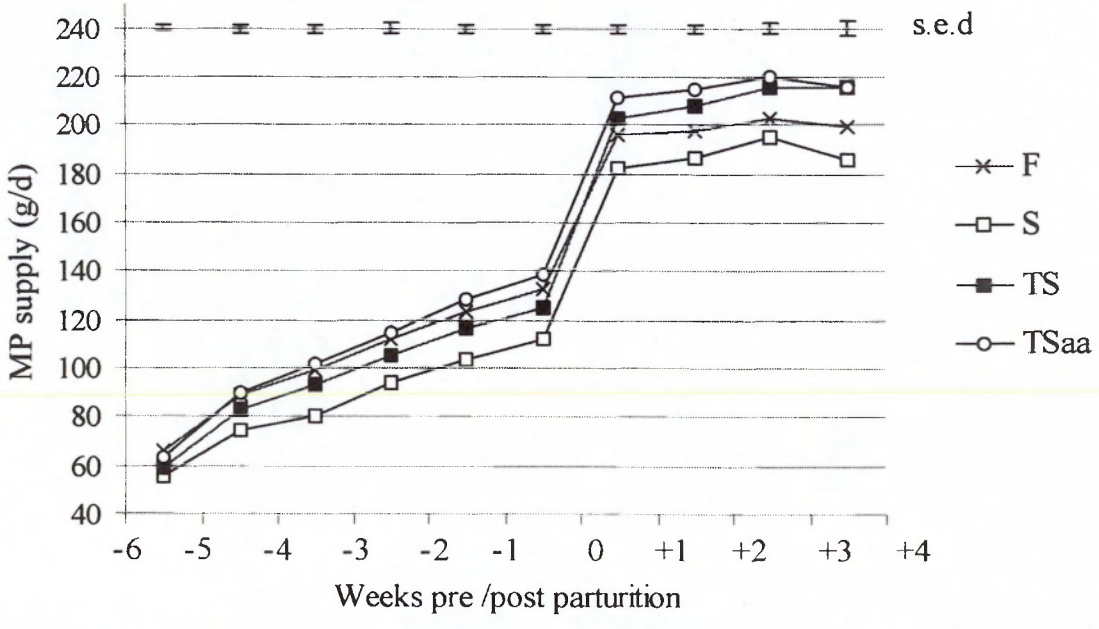

Figure 5.5 Calculated intake of metabolisable protein (MP; g/d) in ewes fed concentrate containing (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) and formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

5.3.4 Ewe weight and condition score change

There was no effect of treatment on ewe weight and CS was seen at 6 weeks *pre partum*, 1 week *pre partum*, immediately *post partum* or at 4 weeks *post partum*. In addition, there was no effect of dietary treatment on *pre partum* or *post partum* weight and CS change (Table 5.7).

Table 5.7 *Weight and condition score (CS) at 6 and 1 week pre partum, immediately post parturition and at 4 weeks post partum(kg), and pre partum (six to 1 week pre partum) and post partum (lambing to four weeks post lambing) weight (kg/week) and CS (units/week) change of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Pre partum weight:-						
At 6 weeks *pre partum*	75.8	75.6	78.2	76.8	1.87	NS
At 1 week *pre partum*	84.2	81.9	84.1	84.1	1.90	NS
Pre partum change	8.4	6.3	5.9	7.3	1.15	NS
Pre partum CS:-						
At 6 weeks *pre partum*	3.87	3.77	3.64	3.56	0.183	NS
At 1 week *pre partum*	3.55	3.55	3.39	3.22	0.180	NS
Pre partum change	-0.32	-0.23	-0.25	-0.34	0.180	NS
Post partum weight:-						
Immediately *post partum*	73.6	70.5	72.7	73.2	1.64	NS
At 4 weeks *post partum*	68.7	63.9	70.0	67.6	2.44	NS
Post partum change	-5.0	-6.7	-2.7	-5.6	1.56	NS
Post partum CS:-						
Immediately *post partum*	3.04	2.77	2.87	2.65	0.191	NS
At 4 weeks *post partum*	2.62	2.52	2.62	2.34	0.187	NS
Post partum change	-0.43	-0.25	-0.25	-0.31	0.143	NS

5.3.5 Colostrum production

5.3.5.1 Yield of Colostrum

There was no significant effect of protein source on the initial yield, secretion rate between 12 - 16 hours *post partum* or on the calculated 24 hour yield of colostrum (Table 5.8).

Table 5.8 *Initial yield of colostrum (g), subsequent secretion rate (12-16h; g/hour) and calculated 24 hour colostrum yield (g) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Initial yield	541	596	436	428	162.5	NS
Secretion rate	78	98	102	101	12.1	NS
24 hour yield	2343	2779	2787	2765	357.9	NS

5.3.5.2 Colostrum composition and component yield at parturition

Ewes fed concentrate containing formaldehyde treated soya-bean meal and amino acids (TSaa) tended to have a higher concentration of fat in the colostrum present at birth compared to ewes fed diets containing formaldehyde treated soya-bean meal without amino acids (TS; P=0.095; Table 5.9). However, there was no difference in the total yield of fat in the colostrum. There was also no effect of diet on the initial concentration or yield of DM, CP, lactose, ash or IgG in the initial secretion of colostrum.

Table 5.9 *Initial concentration (g/kg) and yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Concentration (g/kg)						
DM	375	384	361	394	23.0	NS
CP	184	188	185	205	12.9	NS
Fat	121	128	105	135	11.8	NS
Lactose	15	13	14	10	3.6	NS
Ash	11.7	12.2	12.3	12.3	0.39	NS
IgG	88	77	85	71	12.2	NS
Yield (g)						
DM	207	195	160	180	55.3	NS
CP	98	91	78	92	23.7	NS
Fat	68	67	48	61	19.3	NS
Lactose	7.9	7.4	6.3	4.1	4.02	NS
Ash	6.1	6.4	5.6	5.6	1.79	NS
IgG	46	35	34	37	10.1	NS

5.3.5.3 Colostrum composition and component yield at 16 hours post partum

At 12-16 hours *post partum,* ewes fed diet TSaa produced colostrum that had a higher concentration of fat ($P<0.05$) and CP ($P= 0.069$) and a greater yield of fat ($P<0.05$), CP ($P<0.05$) and DM ($P=0.091$) than ewes fed diet F (Table 5.10).

Table 5.10 *Concentration (g/kg) and yield (g/h) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) in colostrum secreted between 12 and 16 hours post partum from ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Concentration (g/kg)						
DM	239	250	239	281	20.5	NS
CP	64	69	68	87	9.0	NS
Fat	109	120	114	141	10.8	*
Lactose	52	48	45	40	7.3	NS
Ash	8.9	9.2	9.3	9.1	0.33	NS
IgG	18.7	18.3	18.6	26.6	4.92	NS
Yield (g/h)						
DM	18.4	24.9	25.0	28.5	3.83	NS
CP	4.9	6.9	7.2	9.0	1.27	*
Fat	8.5	12.1	11.9	14.2	1.93	*
Lactose	4.1	4.7	4.6	4.0	0.46	NS
Ash	0.7	0.9	1.0	0.9	0.12	NS
IgG	1.4	1.8	2.1	2.8	0.55	NS

5.3.5.4 Calculated yield of constituents over the first 24 hour post partum

The calculated 24 hour yield of DM, CP, fat, lactose, ash and IgG is presented in Table 5.11.

There was no effect of dietary treatment on any of the parameters measured.

Table 5.11 *Calculated colostrum yield (g) of dry matter (DM), crude protein (CP), fat, lactose, ash and immunoglobulin G (IgG) during the first 24 hours post partum from ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
DM	632	771	721	794	106.9	NS
CP	212	250	238	280	35.6	NS
Fat	263	347	315	369	50.4	NS
Lactose	103	115	110	93	15.2	NS
Ash	22.1	27.3	27.1	26.1	3.44	NS
IgG	79.4	82.1	77.8	87.9	12.71	NS

5.3.6 Milk yield

5.3.6.1 Yield of milk, concentration of milk constituents and yield of milk constituents at 7 days post partum

There was no significant effect of protein type on the total milk yield of ewes at 7 days *post partum* (Table 5.12). In addition, there was no significant effect of dietary treatment on the concentration or yield of fat, protein and SNF in milk produced by ewes. Ewes fed diets containing fishmeal (F) tended to produce milk at 7 days *post partum* with a higher concentration of lactose than ewes fed diets containing soya-bean meal (S; P=0.061), but this did not result in a higher lactose yield (g/h).

Table 5.12 *Secretion rate of milk (g/h), concentration of milk constituents(g/l) and secretion rate of milk constituents (g/h) at 7 days post partum from ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Secretion rate of milk (g/h)						
	120	118	116	122	6.9	NS
Concentration of milk constituents (g/l)						
Fat	109.8	110.1	109.7	105.4	8.07	NS
Protein	34.9	35.2	35.0	34.9	0.48	NS
SNF	89.8	88.4	88.9	88.4	1.21	NS
Lactose	47.2	44.7	45.9	45.8	0.86	NS
Yield of milk constituents (g/h)						
Fat	13.4	13.1	12.9	12.9	1.32	NS
Protein	4.3	4.1	4.1	4.2	0.23	NS
SNF	11.0	10.3	10.4	10.7	0.58	NS
Lactose	5.8	5.3	5.4	5.6	0.35	NS

5.3.6.2 Yield of milk, concentration of milk constituents and yield of milk constituents at 21 days post partum

The mean secretion rate of milk obtained from ewes at 21 days *post partum* was 107 g/h and was lower than the secretion rate of 119 g/h produced at 7 days *post partum.* There was no significant effect of protein type on the total milk yield of ewes at 21 days *post partum* (Table 5.13). In addition, there was no significant effect of dietary treatment on the concentration or total yield of fat, protein and lactose in milk produced by ewes at 7 days *post partum*. Ewes fed diets containing formaldehyde treated soya-bean meal (TS) did tend to produce milk with a higher concentration of SNF than ewes fed diets containing soya-bean meal (S; P=0.077), but this did not affect the SNF yield.

Table 5.13 *The total yield of milk (g/h), concentration of milk constituents(g/l) and yield of milk constituents (g/h) at 21 days post partum from ewes which were fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Secretion rate of milk (g/h)						
	99	106	117	106	8.2	NS
Concentration of milk constituents (g/l)						
Fat	116.7	111.0	105.8	102.5	8.58	NS
Protein	34.3	33.9	34.2	34.3	0.32	NS
SNF	88.5	86.7	89.2	87.6	0.98	NS
Lactose	48.2	48.1	49.0	47.3	0.82	NS
Yield of milk constituents (g/h)						
Fat	11.6	11.7	12.5	11.0	1.45	NS
Protein	3.4	3.6	4.0	3.6	0.28	NS
SNF	8.8	9.2	10.4	9.3	0.70	NS
Lactose	4.8	5.1	5.7	5.0	0.39	NS

5.3.7 Litter birth weight and lamb growth rate

There was no effect of dietary protein source on the litter birth weight (Table 5.14). However, ewes fed concentrate containing formaldehyde treated soya-bean meal and added amino acids (TSaa) had lambs which grew quicker and were heavier at 28 days of age than those fed fishmeal (F; $P<0.05$).

Table 5.14 *Litter birth weight (kg), 28 day weight (kg) and lamb growth rate (g/d) of lambs from ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) and formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Litter birth weight	8.04	8.90	8.22	8.93	0.465	NS
28 day litter weight	21.1	22.4	22.4	24.4	0.96	*
Lamb growth rate (0-28 days)	241	261	257	272	10.2	*

5.3.8 Ewe blood metabolites

Mean *pre partum* concentrations of NEFA, BHB and glucose were 0.44, 0.72 and 2.40 mmol/l respectively (Table 5.15). Corresponding *post partum* concentrations were 0.45, 0.67 and 3.12 mmol/l respectively. Ewes fed the concentrate containing soya-bean meal (S) had a significantly higher mean *pre partum* plasma concentration of BHB than those fed fishmeal (F) or formaldehyde treated soya-bean meal (TS; $P<0.05$) and tended to have a higher mean *post partum* plasma concentration of BHB than ewes fed fishmeal (F; $P=0.051$). There was no effect of diet on the *pre partum* or *post partum* concentrations of plasma NEFA or glucose.

Mean *pre partum* concentrations of plasma urea-N, albumin and total protein were 6.4 mmol/l, 35 g/l and 65 g/l respectively (Table 5.15). Corresponding *post partum* concentrations were 7.3 mmol/l, 31 g/l and 64 g/l respectively. There was no effect of diet on the *pre partum* or *post*

partum plasma concentrations of urea-N, albumin or total protein.

Table 5.15 *Pre partum and post partum plasma concentrations of NEFA (mmol/l), BHB (mmol/l), glucose, urea-N (mmol/l), albumin (g/l) and total protein (g/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Mean Pre partum:						
NEFA	0.34	0.48	0.44	0.48	0.070	NS
BHB	0.54	0.97	0.62	0.75	0.124	*
Glucose	2.46	2.36	2.37	2.35	0.098	NS
Urea-N	6.41	6.32	6.51	6.28	0.309	NS
Albumin	34.8	34.7	34.6	35.0	0.78	NS
Total Protein	66.1	63.6	65.0	65.7	1.18	NS
Mean Post partum:						
NEFA	0.46	0.47	0.41	0.47	0.080	NS
BHB	0.58	0.82	0.56	0.73	0.103	NS
Glucose	3.10	3.11	3.11	3.14	0.085	NS
Urea-N	7.33	7.32	7.11	7.33	0.388	NS
Albumin	31.4	31.4	31.5	31.3	1.23	NS
Total Protein	65.3	63.2	64.6	64.4	2.12	NS

5.3.8.1 Ewe blood metabolic profiles

The mean concentration of plasma NEFA decreased from 0.55 mmol/l at 5 weeks *pre partum* to 0.38 mmol/l at 1 week *pre partum* (Figure 5.6). In lactation, plasma NEFA concentrations increased from 0.45 mmol/l in the first week to 0.49 mmol/l during the second week of lactation and then subsequently decreased to 0.39 mmol/l during the fourth week of lactation.

There was no significant effect of diet on the concentration of plasma NEFA at 5 weeks, 3 weeks or 1 week *pre partum*. However, at 2 weeks *pre partum*, ewes fed concentrate containing fishmeal had a significantly lower plasma NEFA concentration than ewes fed the concentrates containing soya-bean meal (S) or formaldehyde treated soya-bean meal with added methionine (TSaa; $P<0.05$). There was no other effect of dietary treatment on the concentration of plasma NEFA.

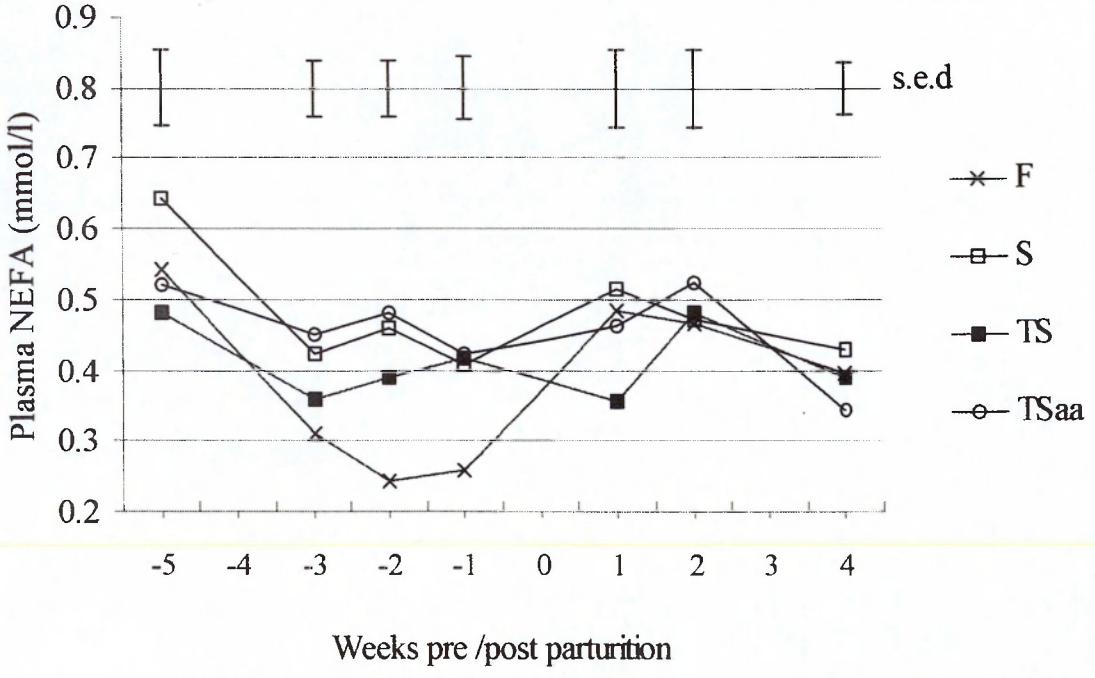

Figure 5.6 Concentrations of plasma NEFA (mmol/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

The concentration of plasma BHB increased from 0.60 mmol/l at 5 weeks *pre partum* to 0.80 mmol/l at 3 weeks *pre partum* and then decreased to 0.72 mmol/l at 1 week *pre partum*. In lactation the mean concentration of plasma BHB increased from 0.55 mmol/l in the first week to 0.75 mmol/l during the second week of lactation and then decreased to 0.69 mmol/l during the fourth week of lactation (Figure 5.7). Ewes fed the concentrate containing soya-bean meal (S) had a higher concentration of plasma BHB than ewes fed all other diets throughout the trial period. This difference was significant at three weeks *pre partum* ($P<0.05$) and tended to be higher than ewes fed diet F at week 2 ($P=0.062$) and week 1($P=0.059$) *pre partum*. There was no significant difference in the weekly concentration of *post partum* plasma BHB.

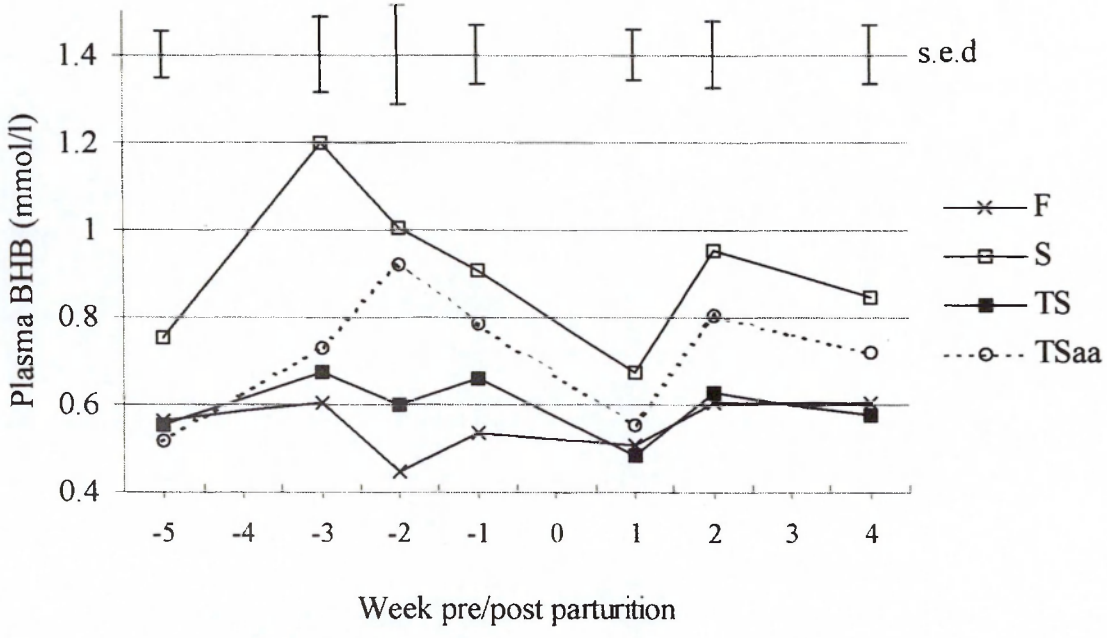

Figure 5.7 Concentrations of plasma BHB (mmol/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

Mean plasma glucose concentrations were higher in all weeks of lactation compared to pregnancy (Figure 5.8). The concentration of plasma glucose was relatively constant in ewes fed all treatments during pregnancy, with mean concentrations of plasma glucose at weeks 5, 3, 2 and 1 *pre partum* of 2.39, 2.44, 2.28 and 2.44 mmol/l respectively. In lactation, mean concentrations of plasma glucose increased from 2.95 mmol/l in the first to 3.39 mmol/l during the third week of lactation. There was no significant effect of diet on the plasma concentration of glucose during pregnancy or lactation.

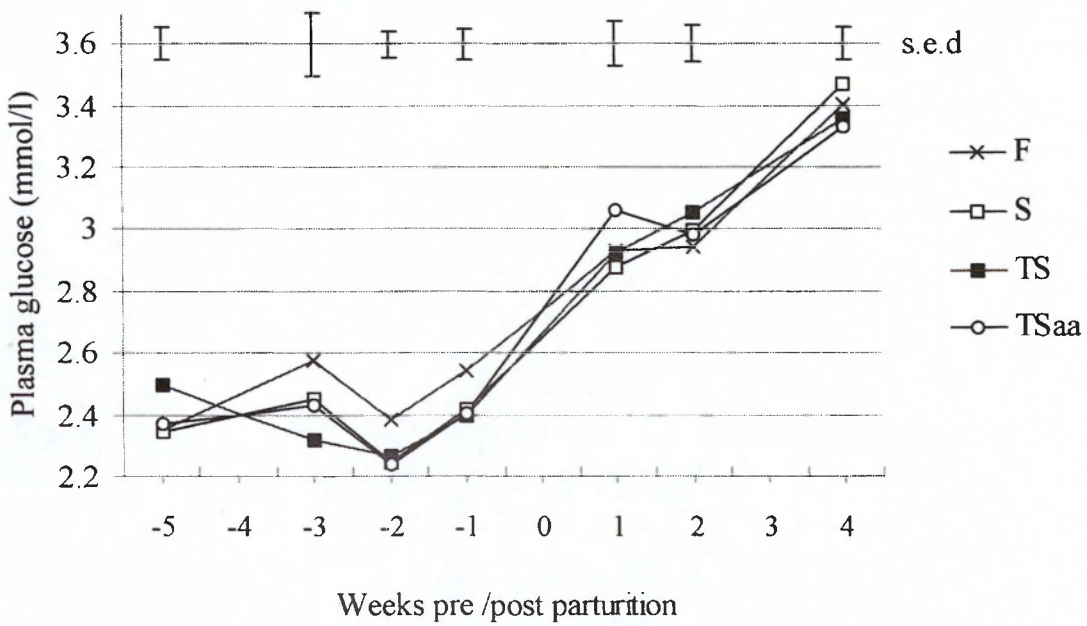

Figure 5.8 Concentrations of plasma glucose (mmol/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

The concentration of plasma urea-N decreased in ewes fed all treatments from week 5 (overall mean of 7.8 mmol/l) to week 1 (5.7 mmol/l) *pre partum* (Figure 5.9). In lactation, mean plasma urea-N concentrations increased from 7.0 mmol/l at 1 week *post partum* to 7.6 mmol/l at 4 weeks *post partum*. There was no significant effect of dietary treatment on the *pre partum* or on the *post partum* plasma urea-N concentration. However, ewes fed diets F and TSaa tended to have higher plasma urea-N concentrations than those fed diets S or TS at week 2 *post partum* (P=0.067).

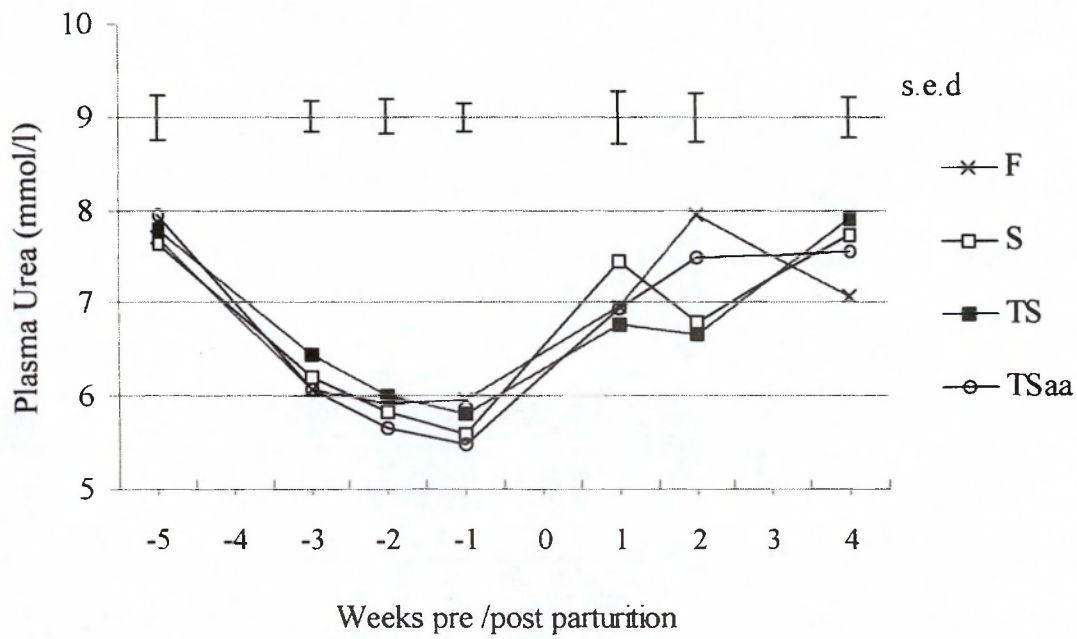

Figure 5.9 Concentrations of plasma urea (mmol/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

The concentration of plasma albumin decreased on all treatments from week 5 (overall mean of 39.5 g/l) to week 1 (29.7 g/l) *pre partum* (Figure 5.10). In lactation, mean plasma albumin concentrations increased slightly from 30.7 g/l at 1 week *post partum* to 31.5 g/l at 4 weeks *post partum* (Figure 5.9). There was no effect of treatment on either the *pre partum* or *post partum* plasma albumin concentration.

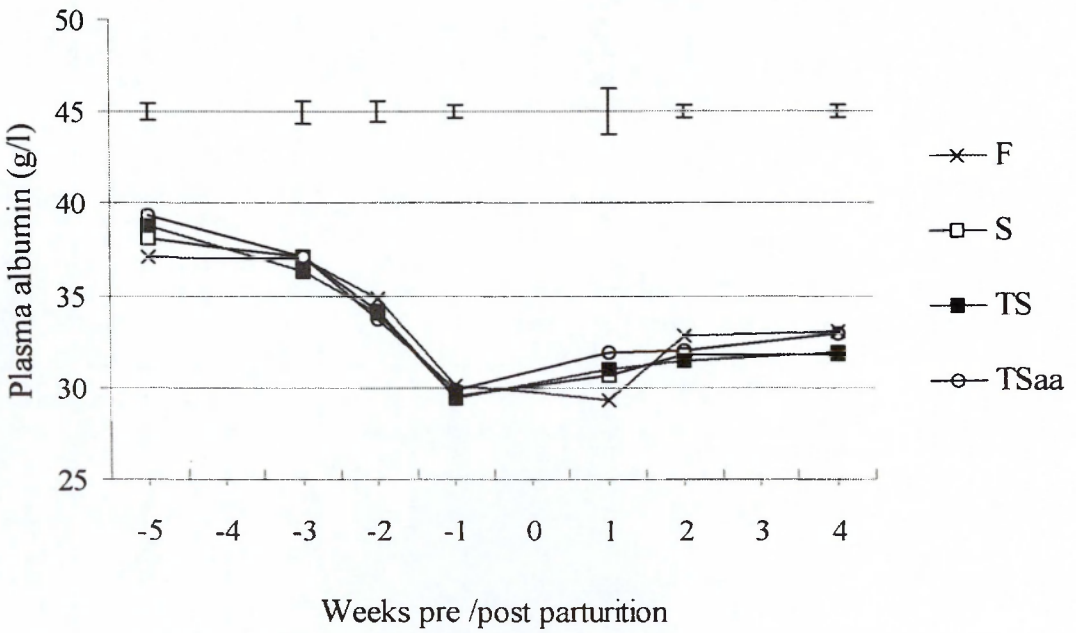

Figure 5.10 Concentrations of plasma albumin (g/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) or formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

The concentration of plasma total protein decreased on all treatments from week 5 (overall mean of 75.8 g/l) to week 1 (58.7 g/l) *pre partum* (Figure 5.11). In lactation, plasma total protein concentration increased from 63.4 g/l at 1 week *post partum* to 65.1 g/l at 4 weeks *post partum*. There was no effect of treatment on the concentration of plasma total protein in ewes during the experimental period.

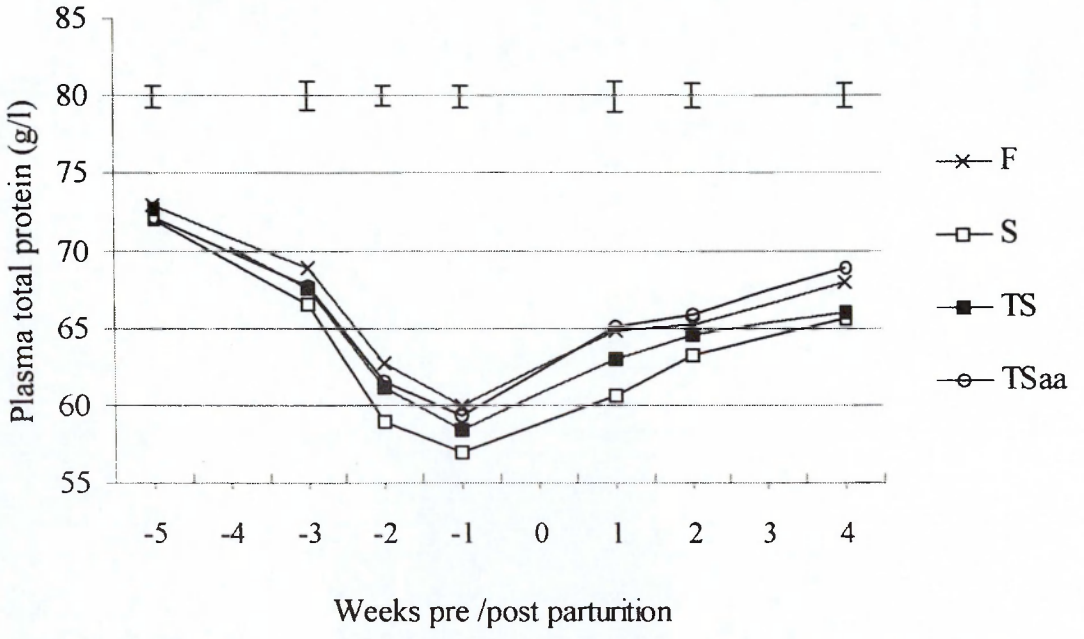

Figure 5.11 Concentrations of plasma total protein (g/l) of ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) and formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation

5.4 DISCUSSION

5.4.1 Summary of main results

Ewes fed formaldehyde treated soya-bean meal with added methionine produced colostrum between 12 and 16 hours *post partum* with a higher concentration of fat and a higher yield of fat and CP than ewes fed fishmeal ($P<0.05$). Ewes fed fishmeal had a lower *pre partum* concentration of plasma BHB compared to ewes fed diets containing soya-bean meal or formaldehyde treated soya-bean meal. Ewes fed formaldehyde treated soya-bean meal with added methionine also had lambs which grew quicker and were heavier at 28 days of age than those fed fishmeal ($P<0.05$).

5.4.1 Protein supply

The high readily soluble N fraction (*a*) of the concentrate containing fishmeal (F) compared to concentrates S, TS or TSaa was not unexpected and is well documented. In addition, fishmeal is regarded as a source of undegradable protein (Gonzalez *et al.*, 1982). However, in the current experiment, the concentrate containing fishmeal (F) had a higher rate of N degradation (*c*) of the potentially degradable N fraction (*b*) than concentrate TS or TSaa. Robinson (1987) reported that individual samples of fishmeal can vary dramatically in their pattern and extent of degradation in the rumen and may account for the high rate of N degradation in diet F observed in the current experiment. The reduction in ruminal N degradability of the soya-bean meal by formaldehyde treatment (S *v.* TS) observed in the current experiment was also observed when rapeseed meal and field beans were treated with formaldehyde (Section 4.3.2) and has been well documented by other authors (Hadjipanayiotou and Photiou, 1995; Rodehutscord *et al.*, 1999; Witt *et al.*, 1999a). In agreement with the current study, Subuh *et al.* (1994) found that the reduction in degradability of rapeseed meal (RSM) and soya-bean meal (SBM) treated with 2.4 g/kg of formaldehyde was caused by a reduction in both the

readily soluble N fraction (*a*) and in the rate of N degradation (*c*) of the potentially degradable N (*b*) fraction (Table 5.16).

Table 5.16 *The effect of treating rapeseed meal and soya-bean meal with 2.4 g/kg of formaldehyde on the readily soluble N fraction (a) and the rate of N degradation (c) of the potentially degradable N fraction (b)*

	Untreated RSM	Treated RSM	Untreated SBM	Treated SBM
a	0.191	0.090	0.119	0.058
b	0.772	0.903	0.875	0.942
c	0.077	0.019	0.090	0.019

From Subuh *et al.* (1994).

In the current experiment, the inclusion of formaldehyde treated soya-bean meal compared to untreated soya-bean meal increased the calculated supply of DUP and MP. Increases in the calculated supply of MP due to formaldehyde treatment is in agreement with experiments by Rodehutscord *et al.* (1999), and similar increases in calculated DUP and MP supply were observed when rapeseed meal and field beans were formaldehyde treated and included in concentrates fed to late pregnant and lactating ewes (Section 4.3.3).

The readily soluble N fraction (*a*) and the rate of N degradation (*c*) of the potentially degradable N (*b*) fraction was similar in concentrates TS and TSaa. This was not unexpected, since the addition of 0.75 g/kg of Smartamine™ M, (Rhône Poulenc, France) is unlikely to change the degradability of the whole concentrate. Although the degradability of concentrates TS and TSaa were similar, ewes fed diet TSaa did have a higher intake of DUP and MP supply both *pre partum* and *post partum* than those fed diet TS, an effect that can be attributed to the higher straw intake of ewes fed this diet.

5.4.2 Ewe and lamb performance

5.4.2.1 Straw intake

The mean straw intake in ewes fed all diets was lower during pregnancy than lactation. This is consistent with the reductions in feed intake during late pregnancy documented by other authors (Robinson, 1987). The basal forage used in the current experiment was winter barley straw and would have a sub-optimal MP:ME ratio (AFRC, 1993). Therefore, the higher straw intake observed when ewes were fed diets F and TSaa, compared to diets S and TS, may have been in response to an improved balance of amino acids or because of an increase in total amino acid supply to the small intestine. The latter mechanism would be consistent with the observations made in Section 4.3.3, where the straw intake in ewes fed formaldehyde treated protein sources was higher than in ewes fed concentrates containing untreated sources. However, in the current experiment, the lack of an intake response in ewes fed diet TS would imply that the amino acid balance of the DUP is more important than the absolute supply of MP. Silva and Ørskov (1988) found that male sheep supplemented with fishmeal had an increased intake of barley straw compared to those receiving similar levels of crude protein from soya-bean meal. They concluded that the effect of fishmeal on straw intake was mediated through an increase in the amino acid supply to the host animal, rather than on rumen changes in the degradation of fibre. Given the observations of Silva and Ørskov (1988), the extent of the intake response would be dependant on the most limiting essential amino acid within the MP as well as the absolute supply of MP (Newbold, 1994). The amino acid supply from fishmeal is usually considered to be superior to that of vegetable proteins (Ngongoni *et al.* 1989), whilst methionine is usually considered to be the first limiting amino acid in soya-bean meal (Lynch *et al.*, 1991; Rodehutscord *et al.*, 1999). It is therefore likely that the superior balance of amino acids in diets F and TSaa may explain the higher intake of straw by ewes on these treatments compared to those fed diet TS.

4.4.2.2 Colostrum production

The initial colostrum yields on all treatments were in agreement with figures quoted by other authors for ewes of similar breeds (Pattinson *et al.*, 1995). It has been well documented that in late pregnancy, when the deficit between ME requirements and ME intake increases there is a need to supply increasing amounts of DUP (Robinson, 1987). There is also evidence to show that when the protein source fed in late pregnancy is largely rumen undegradable, increases in initial colostrum yield may occur (Robinson, 1987). However, in agreement with data published by Dawson *et al.* (1999), the initial colostrum yield in the current experiment was not affected by protein source or DUP supply. In the experiment of Dawson *et al.* (1999), increasing the UDP supplied to twin-bearing ewes from 24.0 g/d to 49.8 g/d during the 6 weeks prior to lambing had no effect on the initial colostrum yield or the quality of that colostrum. The absence of a difference in the current experiment and also that of Dawson *et al.* (1999) may be due to the higher ME intake of these ewes (mean ME intakes of 12.5 and 14.5 MJ ME/d respectively). In addition, initial colostrum yield is particularly variable between individual sheep (see section 3.4.2.2), making treatment differences more difficult to identify (Pattinson *et al*, 1995). In the current experiment, the coefficient of variation for the initial colostrum yield was much greater than that for the secretion rate of colostrum between 12-16 hours *post partum* (69 and 31 % respectively).

In agreement with the experiments reported in Chapters 3 and 4, the mean concentrations of DM, CP, fat ash and IgG in colostrum were lower, and the concentration of lactose was higher, in the colostrum secreted between 12 and 16 hours *post partum*, compared to colostrum present at birth. At 12-16 hours *post partum*, ewes fed diet TSaa had a significantly higher yield of total protein and fat than ewes fed diets F, S or TS. This could be due to an increased total supply of amino acids or an improved balance of amino acids available to the mammary gland.

However, the lack of an increase in the yield of CP or fat in ewes fed diet TS suggests that the addition of rumen-protected methionine to formaldehyde treated soya-bean meal is necessary to increase the yield of these constituents. Unfortunately, the experimental design does not allow conclusions to be drawn on whether it is solely the addition of protected methionine which is causing the effect or whether it is a combination of formaldehyde treatment and the addition of rumen protected methionine. In order to answer this question it would have been necessary to have an additional dietary treatment containing untreated soya-bean meal with rumen-protected methionine. Robinson (1990a) noted that microbial protein is likely to be deficient in cystine, and the formation of cystine from methionine in late pregnant ewes may also cause methionine to become limiting. Rossi *et al.* (1999) noted that this may be particularly noticeable in diets where the main source of DUP was also low in methionine, such as soya-bean meal in the current experiment. Sevi *et al.* (1998) reported beneficial effects of feeding rumen-protected methionine and lysine to lactating ewes. In the experiment of Sevi *et al.* (1998), ewes receiving the amino acid supplemented diet had a higher yield of milk, milk protein, milk fat and milk lactose and had a higher gross efficiency of utilisation of dietary nitrogen than those fed an un-supplemented diet. Rodehutscord *et al.* (1999) reported that formaldehyde treatment of lupins caused reductions in the availability of sulphur-containing amino acids for wool growth in Merino wethers, and therefore production responses to feeding rumen protected methionine are likely to be greater when the protein source fed has been formaldehyde treated.

5.4.2.3 Litter birth weight

It is apparent from the study of Robinson and McDonald (1989) that ewes at maintenance will increase lamb birth weight in response to switching to proteins that are resistant to degradation in the rumen. In the current experiment the small difference in *pre partum* MP supply (103, 87,

97 and 106 g/d for ewes fed diets F, S, TS and TSaa respectively) did not cause a difference in lamb birthweight. In agreement with the current work, Dawson *et al.* (1999) failed to observe any a difference in lamb birth weight when the diets fed during late pregnancy had a larger range of calculated MP supply than that in the current experiment (126 to 177 g/d; Dawson *et al.*, 1999). In Chapter 4, no difference was seen in litter birth weight when untreated and formaldehyde treated field beans were fed to ewes in late pregnancy, producing MP intakes of 116 and 131 gMP/d for untreated and treated protein sources respectively (section 4.3.7). In an experiment by McNeill *et al.* (1997), twin-bearing ewes fed 141 gCP/d had higher N accretion in the gravid uterus than ewes fed 81 gCP/d. However, the magnitude of the response was relatively small and was apparently buffered by the mobilisation of N from maternal tissues (McNeill *et al.*, 1997). No further increase in N accretion in the gravid uterus occurred in ewes fed 157 gCP/d. The authors concluded that maternal body tissue is a major source of mobilised N in ewes fed below their predicted crude protein requirements and the site of increased N accretion in ewes fed above predicted requirements. Any effects of MP supply in the current experiment could therefore have been masked by the buffering effect of maternal tissue protein stores. McNeill *et al.* (1997) also hypothesised that ewes fed the low protein diet could have more efficient recycling of urea and possibly reduced hepatic catabolism of amino acids. Feeding high protein diets to ewes in late pregnancy is also likely to result in a significant proportion of absorbed amino acids being deaminated (McNeill *et al.*, 1997).

5.4.2.4 Milk production and lamb growth rate

In contrast to the positive effects of feeding diet TSaa on intake of barley straw and component yield of colostrum at 12-16 hours *post partum*, there was no similar effect on the total yield of milk or on the yield of milk fat or protein at 7 or 21 days *post partum*. During early lactation rumen outflow rates are high and DUP will make a larger contribution to the overall protein

supply of the animal than during pregnancy. Consequently, any effect of an amino acid deficiency within the DUP would be more apparent at these higher outflow rates. It might therefore be expected to see a response in the yield or composition of milk from ewes fed methionine supplemented diets. Despite the apparent absence of a dietary effect on milk and compositional yield, ewes fed the concentrate containing formaldehyde treated soya-bean meal with added rumen protected methionine (TSaa) did have lambs with a higher liveweight gain (birth to 28 days of age) than those fed concentrate containing fishmeal (F), a result which may question the validity of the technique used for measuring milk yield. It is possible that, in the current experiment, methionine supplementation caused an improvement in the amino acid profile of the milk and improved the biological value of the milk protein for the suckling lambs. Several studies have reported that dietary supplementation of amino acids will cause increases in the corresponding plasma concentration (Papas *et al.*, 1984; Rogers *et al.*, 1987), whilst Lynch *et al.* (1991) reported a higher calculated daily intake of methionine and lysine in suckling lambs when the maternal diets contained encapsulated lysine and methionine. Alternatively, the higher growth rates observed in lambs from ewes fed diet TSaa may be due to the improved colostrum quality from ewes fed this diet. However, this is an unlikely explanation, as lambs from ewes fed diet TSaa only had significantly higher growth rates from days 14-28 of lactation (Table 5.17).

Table 5.17 *Lamb growth rate (g/d) from ewes fed concentrate containing fishmeal (F), soya-bean meal (S), formaldehyde treated soya-bean meal (TS) and formaldehyde treated soya-bean meal with added methionine (TSaa) during late pregnancy and early lactation*

	Treatment means					
	F	S	TS	TSaa	s.e.d.	Significance
Lamb growth rate from:-						
Birth to 7 days	279	264	253	291	19.0	NS
7 to 14 days	271	290	300	265	21.7	NS
14 to 21 days	213	250	224	273	19.7	*
21 to 28 days	207	235	223	278	20.2	*

5.4.2.5 Ewe weight and condition score change

There was no significant difference in the *pre partum* or *post partum* condition score or weight change of ewes. However, ewes fed the concentrate containing untreated soya-bean meal had a higher mean *pre partum* plasma concentration of BHB. Lynch and Jackson (1983) found that ewes fed diets with a high concentration of CP had higher plasma BHB concentrations compared to those ewes fed a diet low in CP (0.43 *v.* 0.30 mmol/l respectively). In such cases, improvements in dietary protein supply cause increases in adipose tissue mobilisation resulting in increased plasma BHB concentrations. However, in the current experiment there was no evidence from the changes in CS or ewe weight or from plasma BHB or NEFA concentration that improvements in the calculated MP supply by formaldehyde treatment, or the improvement in the relative value of the protein by addition of methionine led to increases in adipose mobilisation. Everts (1990) reported a negative correlation in pregnant ewes between the ME intake and the plasma BHB concentration. In the current experiment, ewes fed diet S had a low intake of straw compared to ewes fed diets F and TSaa which led to small reductions in *pre partum* and *post partum* ME intake in ewes fed diet S (0.8 and 1.15 MJ ME/d lower in diet S than the mean of F and TSaa in pregnancy and lactation respectively) and may have contributed to the increases in plasma BHB concentration in ewes fed diet S.

5.5 CONCLUSIONS

The results of the current experiment show that reductions in the readily soluble N fraction and the rate of N degradation and increases in the calculated MP supply to pregnant and lactating ewes can be achieved by the treatment of vegetable protein sources with formaldehyde. The addition of rumen protected methionine to formaldehyde treated soya-bean meal (TSaa) increased the intake of straw by pregnant ewes and led to improvements in the yield of CP and fat in colostrum at 12-16 hours *post partum*. Lambs from ewes fed diet TSaa also grew significantly quicker from birth to 28 days of age than those fed diets F. From the current experiment, it appears that formaldehyde treatment of soya-bean meal is only really worthwhile if diets for pregnant and lactating ewes are also supplemented with rumen protected methionine. However, addition of rumen protected methionine to untreated soya-bean meal may have also caused a production response.

CHAPTER 6

THE EFFECTS OF DIETARY PROTEIN SOURCE ON THE METABOLISM AND PERFORMANCE OF EWES IN LATE PREGNANCY AND EARLY LACTATION

6.1 INTRODUCTION

The current series of three experiments were designed to test the hypothesis that fishmeal could be successfully replaced by alternative protein sources in concentrates fed to twin-bearing ewes in late pregnancy and early lactation. The first experiment investigated a product based on formaldehyde treated soya-bean meal, and evaluated the effect of replacing part of the concentrate diet with feedblocks fed *ad libitum*. The results demonstrated that acceptable levels of production could be achieved by replacing fishmeal with a product based on protected soya-bean meal with an improved amino acid balance.

It has become increasing likely that legislation could force the exclusion of fishmeal from ruminant diets (House of Lords, 1996). Therefore, subsequent experiments were concerned with examining alternative protein sources to fishmeal. The second experiment was designed to investigate the effects of including either untreated or formaldehyde treated UK produced protein sources (field beans and rapeseed meal) on the performance and metabolism of twin-bearing ewes. Results from this experiment showed that the fishmeal used resulted in lower levels of production (e.g. lamb growth rate) compared to the other protein sources. In addition, there also appeared to be no benefit, or in some cases a negative effect (e.g. 21-day milk yield), of feeding formaldehyde treated field beans or rapeseed meal compared to feeding them untreated. Other authors have shown that certain amino acids, for example methionine, which is often the first limiting for ewes in late pregnancy and early lactation, can be reduced in formaldehyde treated protein sources, thus lowering the relative value of the protein (Rodehutscord *et al.*, 1999). Soya-bean meal has been cited as being low in methionine

(Schingoethe, 1996) and consequently the third experiment investigated the effects of including untreated soya-bean meal, formaldehyde treated soya-bean meal and formaldehyde treated soya-bean meal with added rumen protected methionine as alternatives to fishmeal in diets fed to ewes in late pregnancy and lactation. Results from this experiment showed that ewes fed the diet containing formaldehyde treated soya-bean meal with added methionine produced lambs with higher growth rates than those fed fishmeal and. In addition, ewes fed formaldehyde treated soya-bean meal with added methionine had a higher straw intake *pre partum* than ewes fed diets containing soya-bean meal or formaldehyde treated soya-bean meal (with no added methionine) and had an improved 12-16 hour yield of colostrum fat and protein than ewes fed diets containing fishmeal.

6.2 EFFECT OF SOURCE AND TREATMENT OF DIETARY PROTEIN

6.2.1 Effects of metabolisable protein supply on animal performance

In the current series of experiments, the calculated mean *pre partum* MP intake for each dietary treatment ranged from 87 g/d to 138 g/d, whilst during the *post partum* period MP intakes ranged from 186 to 283 g/d. The variation in ME intakes in both the *pre partum* (mean 13.8 MJ/d; s.d. 1.29 MJ/d) and the *post partum* (mean 22.5 MJ/d; s.d. 1.03 MJ/d) periods was small. There was no clear relationship between the calculated MP intake *pre partum* and litter birth weight, initial colostrum yield or colostrum secreted between 12 and 16 h *post partum*. These results would suggest that there is little opportunity to manipulate either lamb birth weight or colostrum production by altering MP intake within the range reported here. In agreement with the current results, Pattinson (1992) reported that increasing crude protein intake from 241 to 339 g/d in late pregnancy had no effect on the yield of colostrum at birth or from 12-16 h *post partum*. However, O'Doherty and Crosby (1997) reported that increasing the CP intake of ewes in late pregnancy from 76 to 158 g/d significantly increased colostrum yield at 1 h, 10 h and 18 h *post partum*. In the current series of experiments, crude protein intakes *pre partum*

ranged from 170 g/d (diet S, Chapter 5) to 267 g CP /d (diet FB, Chapter 3). In the experiment by O'Doherty and Crosby (1997) it is likely that ewes receiving the lower intake of crude protein would be particularly deficient in dietary protein and that lower colostrum yields would result. Other authors have also shown no effect of MP intake on lamb birth weight (e.g. Dawson *et al.*, 1999). McNeill *et al.* (1997) reported that there was no difference in the N accretion in the gravid uterus when twin-bearing ewes were fed 157 g CP/d compared to those fed 141 g CP/d and concluded that maternal body tissue is the site of increased N accretion in ewes fed above their protein requirements.

A significant relationship existed between the *pre partum* MP supply and the mean *pre partum* plasma concentration of BHB ($P<0.05$; $r^2 = 37.8$; Figure 6.1). The lower concentrations of BHB at higher MP intakes may suggest that additional amino acids were being used for gluconeogenesis and that this in turn reduced the accumulation of BHB in the plasma. Therefore, feeding diets that are high in MP to ewes in late pregnancy could have a role in the reduction of ketosis in late pregnant ewes.

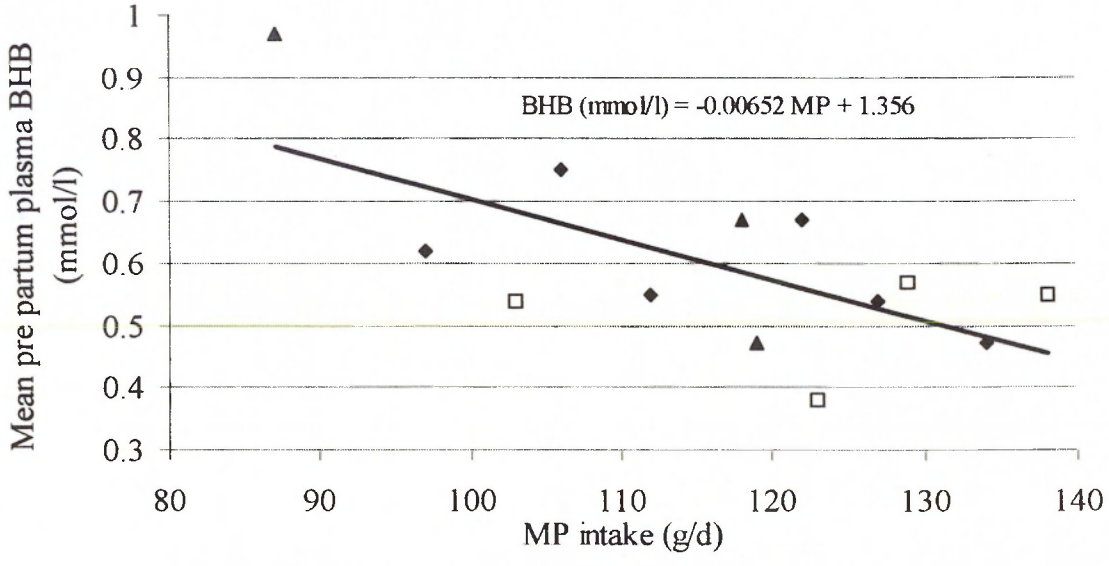

Figure 6.1. The effect of mean *pre partum* MP intake (g/ewe/d) on the mean *pre partum* concentration of plasma BHB (mmol/l) in ewes fed concentrates containing untreated vegetable protein sources (▲), formaldehyde treated protein sources (◆) and fishmeal (□)

The intake of MP by ewes in the *post partum* period, was significantly positively related to their lamb growth rate (g/d) both from birth to 14 days of age ($P<0.01$; $r^2 = 55.6$; Figure 6.2) and from 14 to 21 days of age ($P<0.01$; $r^2 = 56.0$; Figure 6.3).

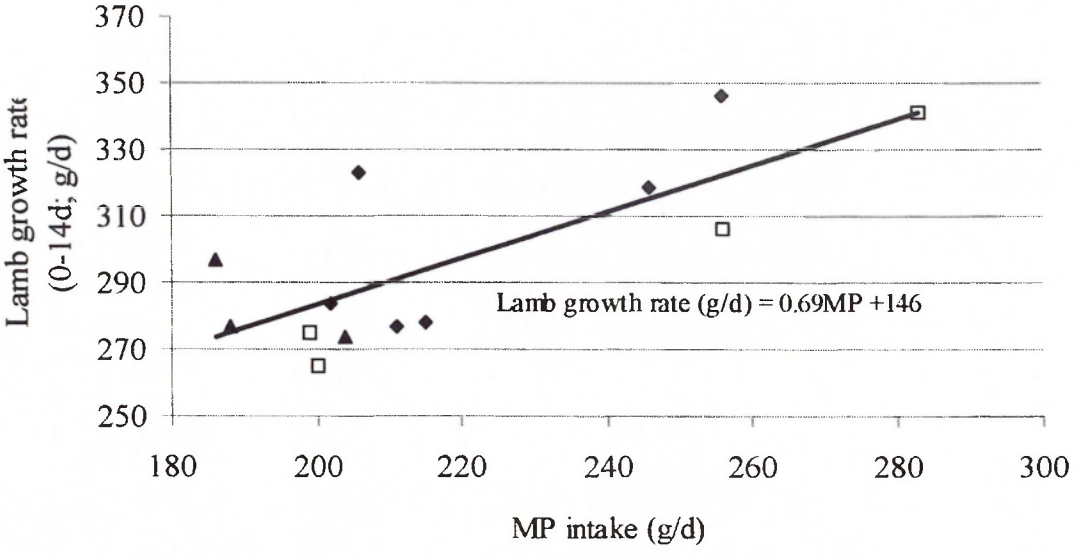

Figure 6.2. The effect of mean *post partum* MP intake (g/ewe/d) on the growth rate of lambs (0-14d; g/d) from ewes fed concentrates containing untreated vegetable protein sources (▲), formaldehyde treated protein sources (◆) and fishmeal (□)

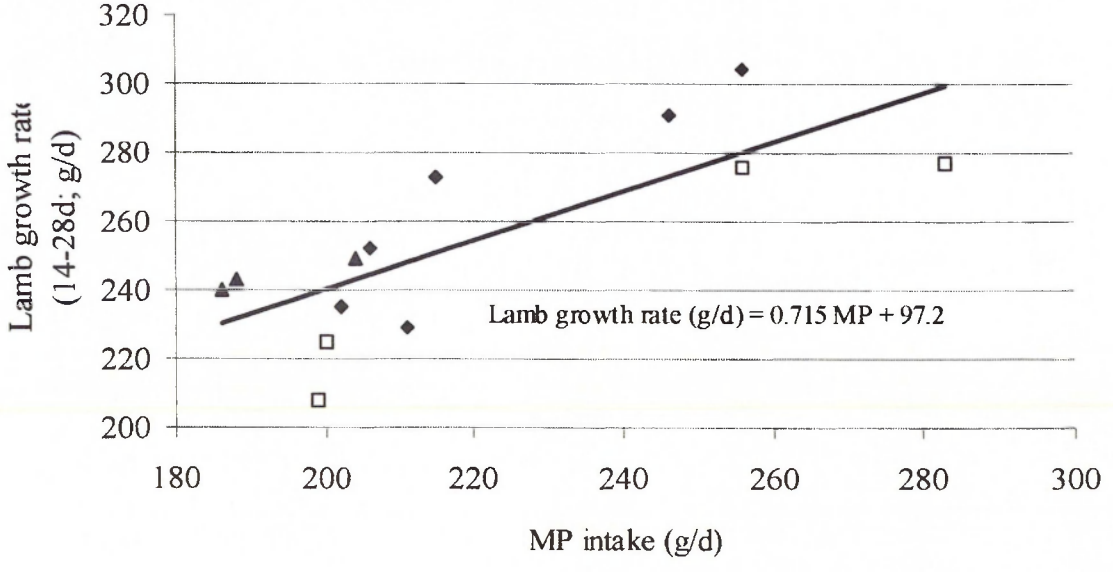

Figure 6.3. The effect of mean *post partum* MP intake (g/ewe/d) on the growth rate of lambs (14-21d; g/d) from ewes fed concentrates containing untreated vegetable protein sources (▲), formaldehyde treated protein sources (◆) and fishmeal (□)

In addition to the significant positive relationship between *post partum* MP intake and the lamb growth rate, there was a significant positive relationship between *post partum* MP intake and mean *post partum* plasma NEFA concentration ($P<0.001$; $r^2 = 65.4$; Figure 6.4). In agreement with other authors (e.g. Sinclair *et al.*, 1994), increases in MP intake increased the rate of body fat mobilisation.

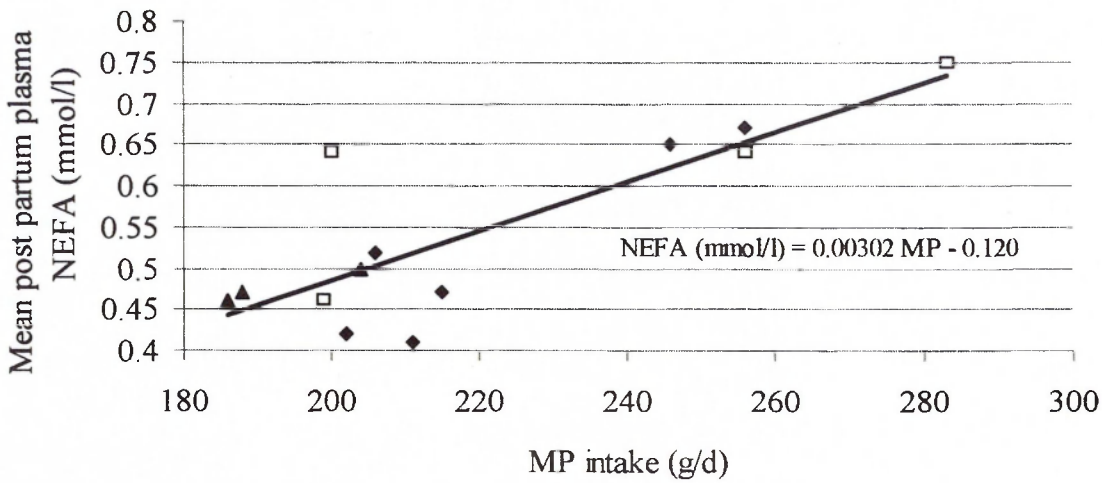

Figure 6.4. The effect of mean *post partum* MP intake (g/ewe/d) on the mean *post partum* concentration of plasma NEFA (mmol/l) in ewes fed concentrates containing untreated vegetable protein sources (▲), formaldehyde treated protein sources (◆) and fishmeal (□)

It is logical to assume that increases in lamb DLWG at high MP intakes were caused by an improved milk supply to the lambs. However, in contrast to the significant positive relationship between *post partum* MP intake and lamb DLWG (g/d), there was no similar relationship between *post partum* MP and milk yield at 7 or at 21 days *post partum* ($P>0.05$). Given these contrasting results, it could be suggested that the method used to quantify the milk yield was not a true reflection of the actual milk yield.

6.2.2 Methods of calculating metabolisable protein supply

Current systems for predicting protein supply in ruminants calculate the MP supply to the small intestine (e.g. INRA, 1989; CSIRO, 1990; AFRC, 1993), albeit by different methods. In the

current series of experiments, AFRC (1993) was used to calculate MP intake. However, alternative systems may have resulted in a different interpretation of the MP intake. Data presented in Table 6.1 compares the MP intake for diets containing fishmeal, field beans or formaldehyde treated field beans (Chapter 4) predicted by both AFRC (1993) and INRA (1989).

Table 6.1 *The predicted intake of microbial crude protein (MCP; g/d), digestible undegradable protein (DUP; g/d) and metabolisable protein (MP; g/d) at two different outflow rates by the systems of AFRC (1993) and INRA (1989) for diets containing 1kgDM of concentrate containing fishmeal (F), field beans (FB) or formaldehyde treated field beans (fFB) and 0.5kgDM of barley straw*

	Diet	MCP (g/d)		DUP (g/d)		MP (g/d)	
		(r) 0.05	0.08	0.05	0.08	0.05	0.08
AFRC (1993)	F	<u>148</u>	145	32	39	130	132
	FB	<u>148</u>	152	23	31	125	128
	fFB	135	121	47	60	133	137
INRA (1989)	F	<u>126</u>	<u>124</u>	40	53	121	127
	FB	<u>129</u>	<u>128</u>	32	46	115	122
	fFB	<u>126</u>	119	56	75	137	145

<u>Underlined</u> values = ruminal fermentable energy supply limited the production of MCP (g/d)

As with any other model of complex biological systems, the MP system (AFRC, 1993) has limitations (Sinclair and Wilkinson, 2000). One disadvantage of the MP system is that the calculation of fermentable energy supply could be an over estimation as no account is made of the UDP content of the diet. For example, Beever and Cottrill (1994) noted that AFRC (1993) defined the FME content of fishmeal as 85 % of its ME value, even though 56 % of ME is in the form of UDP. In the current series of experiments, ewe diets which differed in protein degradability and the supply of rumen undegradable protein were compared. The use of AFRC (1993) in this situation potentially over predicts the total fermentable energy supply to the rumen and in situations where fermentable energy is limiting MCP production, will over predict

the total MP intake to the ewe. This would be a particular problem for diets high in UDP. A major difference between the UK (AFRC, 1993) and the French PDI system (INRA, 1989), is that the latter system reduces the calculated fermentable energy supply (in this case defined as fermentable organic matter; FOM) for the UDP content of the diet.

AFRC (1993) predict the yield of MCP to be greater and the yield of DUP to be smaller than INRA (1989) on all diets and at both ruminal outflow rates (Table 6.1). This is reflected in the calculated MP intake by the two methods, with AFRC (1993) tending to predict higher amounts of MP compared to INRA (1989) in diets containing degradable protein sources (FB) and lower amounts in those containing undegradable sources of protein (fFB). There is, however, overall agreement between AFRC (1993) and INRA (1989) on the effect of formaldehyde treatment on calculated MP intake and on the effect of outflow rate.

As previously mentioned, the system of AFRC (1993) may tend to over predict the MCP and hence the MP intake in diets which contain undegradable protein sources, compared to those containing degradable protein sources. It can be calculated that for ewes fed diets FB and fFB (Chapter 4; fed 1 kg concentrate DM and 0.5kg barley straw DM per day; containing 14.75 MJ of FME/kg DM) the effect of reducing the calculated FME supply for the UDP content of the diet would reduce calculated MP intake in pregnancy by 5.3 g/d and 9.4 g/d for diets FB and fFB respectively.

6.2.2.1 The in situ *technique*

The majority of systems used to predict MP supply to ruminants use the *in situ* technique to estimate protein degradability and it was used in the current experiment to estimate ruminal and post ruminal protein supply. The *in situ* technique has been criticised as having considerable variation in the reported degradability between different laboratories (Madsen and Hvelplund,

1994). Huntington and Givens (1995) cited a number of reasons for such variation including bag pore size, bag material, sample particle size, sample weight:bag surface area ratio, host diet effects, inter animal effects and inter species effects. In addition, the extent of microbial contamination of the bag residues could lead to errors in the estimation of protein degradability (Huntington and Givens, 1995). These effects are particularly true in feeds where the N content of the feed represents only a small proportion of the total dry matter (Varvikko and Lindberg, 1985).

In the current series of experiments, all concentrates were ground through a 2 mm screen and the small particles were removed by hand sieving through a 45 μm sieve to minimise the physical loss of small undegraded particles from the bags. Emanuele and Staples (1988) reported that grinding through a 2 mm screen would give a normal distribution of particle sizes within the sample. The bags used were made from a welded mesh polyester and had a pore size of 40 μm. Welded mesh polyester bags are recommended by AFRC (1992) as this material produces a more consistent pore size than woven material which has a pore size in the range of 35-50 μm. The ratio of sample weight:bag surface area was approximately 10 mg/cm^2 which is within the range of 4-17 mg/cm^2 reported by Huntington and Givens (1995) as acceptable. In the current series of experiments, mature wether sheep were used for all *in situ* work, eliminating any theoretical effects of species and age on rumen degradability discussed by Huntington and Givens (1995). The basal diet was fed at 1.1 x maintenance and was a mean of the composition of those fed in the respective production experiment.

Huntington and Givens (1995) suggested that the *in situ* incubation of feedstuffs is currently the best method of estimating degradation in the rumen as it provides the closest comparison with *in vivo* results. Whilst other methods can be used to estimate the quantity of feed protein

degraded in the rumen, namely solubility techniques, proteolytic enzyme assays (Poos-Floyd, 1985) and by incubating with rumen fluid (Broderick, 1987), they all appear to have inherent disadvantages. Solubility measurements alone do not indicate degradability, and can not predict the rate and extent of protein degradation (Broderick, 1987). Roe *et al.* (1991) found little correlation between degradability coefficients generated by the *in situ* technique and by the *in vitro* proteolytic enzyme assays. Techniques in which feed is incubated with buffered rumen fluid often measure ammonia concentration as an index of proteolytic activity (Husain, 1985). However, the observed concentration depends as much on the utilisation of ammonia by microorganisms as it does on the rate of protein degradation. In addition, accumulation of the end products of degradation may inhibit degradation (Husain, 1985). Techniques which use rumen fluid also require the maintenance of donor animals.

6.2.3 The effect of feeding diets containing fishmeal

Fishmeal is regarded as a suitable source of undegradable protein for ewes in late pregnancy and early lactation (Robinson *et al.*, 1979). In the experiments reported in both Chapter 4 and Chapter 5, concentrates containing fishmeal had a high readily soluble N fraction (*a*) in comparison with concentrates containing vegetable protein sources, a result which is in agreement with that of Witt *et al.* (1999a). In these experiments, concentrates containing fishmeal had a higher rate of N degradation (*c*) of the potentially degradable (*b*) fraction, compared to untreated rapeseed meal and untreated soya-bean meal (Chapter 4 and Chapter 5 respectively). By contrast, in the experiment reported in Chapter 3, concentrates containing fishmeal had a similar readily soluble N fraction (*a*) and a similar rate of N degradation (*c*) of the potentially degradable (*b*) fraction compared to concentrates containing formaldehyde treated soya-bean meal. Mehrez *et al.* (1980) reported that the rate of N degradation in fishmeal was increased with longer periods of storage prior to processing and could account

for the high rumen N degradability observed in the current series of experiments.

In Chapters 4 and 5 there was no overall indication that fishmeal was a superior protein source to untreated vegetable protein sources for pregnant and lactating ewes, whilst in the experiment reported in Chapter 3 ewes fed fishmeal did have lambs with higher growth rates than those fed a soya-bean meal based replacer. The use of fishmeal as a control protein source is somewhat questionable, given the observed variability in the quality of fishmeal used in the current series of experiments and underlines the importance of characterisation of fishmeal prior to its use in experiments designed to compare fishmeal with alternative protein sources.

6.2.4 The effect of protecting vegetable protein sources with formaldehyde

The rate of formaldehyde treatment used in the current series of experiments varied with the protein source being treated. The rate of formaldehyde used was 2.8 g/kg of soya-bean meal, 2.38 g/kg of field beans and 3.8 g/kg of rapeseed meal. By using average values for the crude protein content of soya-bean meal, field beans and rapeseed meal of 500, 315 and 390 g/kgDM respectively, the rates of formaldehyde (g) per 100 g CP can be calculated as 0.56, 0.75 and 0.97 for soya-bean meal, field beans and rapeseed meal respectively. The rate used was chosen to optimise the reduction in rumen protein degradability, without affecting the subsequent intestinal digestibility (J. Twigge, personal communication). These rates of application are within the range of data presented earlier on the effect of formaldehyde treatment on both the rumen degradability (Mir *et al.*, 1984; Hadjipanayiotou, 1992; Subuh *et al.*, 1994 and Woong-Yeoul *et al.*, 1999; Figure 1.6) and on the subsequent digestibility (Antoniewicz *et al.*, 1992; Figure 1.7).

In the experiment reported in Chapter 3, concentrates containing a product based on formaldehyde treated soya-bean meal and amino acids was compared with concentrate

containing fishmeal and therefore did not allow an evaluation of the effects of formaldehyde treatment of vegetable protein *per se*. In Chapters 4 and 5, both untreated and formaldehyde treated protein sources were included. In both of the latter experiments, formaldehyde treatment reduced the calculated effective rumen degradability of N at rumen outflow rates of 0.05 and $0.08h^{-1}$ and is consistent with observations made by other authors (Hadjipanayiotou and Photiou, 1995; Rodehutscord *et al.*, 1999; Witt *et al.*, 1999a). In addition, formaldehyde treatment of vegetable protein sources led to increases in both the *pre partum* and *post partum* supply of MP. However, despite this, ewes fed formaldehyde treated vegetable protein sources did not show improvements in physical performance compared to those fed untreated vegetable protein sources. In Chapter 4, no significant positive effect of including formaldehyde treated field beans or rapeseed meal was observed compared to including untreated sources. In addition, the milk yield at 21 days *post partum* was significantly lower in ewes fed formaldehyde treated compared to untreated protein sources. Similarly, in the experiment reported in Chapter 5, no significant improvement in production was seen when formaldehyde treated soya-bean meal (with no supplemental methionine) was fed. The literature on the effects of feeding formaldehyde treated vegetable protein sources on the performance of ruminants is inconclusive. For example, Huhtanen *et al.* (1991) reported increases in the milk yield of lactating dairy cows fed 1.5 kg of barley distillers grains treated with 1.8 g/kg of formaldehyde, compared to those fed the same amount of untreated grains. Hamilton *et al.* (1992) reported significant increases in the milk protein yield in dairy cows fed sunflower meal treated with 5 g/kg of formaldehyde compared with those fed untreated sunflower meal. In contrast to the results of Huhtanen *et al.* (1991) and Hamilton *et al.* (1992), but in agreement with the current series of experiments, Hadjipanayiotou (1992) reported no improvement in milk yield or constituent yield of milk in lactating Chios ewes fed diets containing soya-bean meal treated with 1.2 g/kg of formaldehyde compared with those fed diets containing untreated soya-bean

meal, whilst Tewatia *et al.* (1995) reported that lactating goats fed diets including faba beans treated with 4.3 g of formaldehyde/kg produced similar yields of milk, but with a lower concentration of CP than ewes fed diets containing untreated faba bean.

There are a number of possible reasons for the absence of a positive effect of formaldehyde treatment on ewe productivity reported in the current work:-

(a). The actual increase in MP supply was not sufficient to cause observed differences in production.

(b). The increase in MP supply due to formaldehyde treatment of protein is lower than AFRC (1993) predicted, as no account of the modifying effect of DUP content of the diet on the rumen fermentable energy supply was considered.

(c). Formaldehyde treatment caused a reduction in the rumen degradable protein supply so that microbial crude protein production was not optimal.

(d). The relative value of the protein reaching the small intestine was lower in ewes fed formaldehyde treated protein sources.

The small difference in MP supply and the effect of including DUP in the calculation of available FME for MCP production has already been considered and will not be discussed further here. There is evidence from the current series of experiments that both points (c) and (d) may render formaldehyde treatment less effective. Taking the two points separately, in the experiment reported in Chapter 4, ewes fed formaldehyde treated protein sources produced a similar yield of milk at 7 days *post partum* as ewes fed untreated protein sources, but produced

a lower yield of milk at 21 days *post partum* when intakes, and hence outflow rates, were relatively high. At these high outflow rates it is possible that microbial protein production was reduced because of the effective lower supply of rumen degradable protein. In the experiment reported in Chapter 5, a higher fat concentration and a higher fat and protein yield in colostrum secreted between 12 and 16 hours *post partum* were seen in ewes fed concentrates containing formaldehyde treated soya-bean meal with added rumen protected methionine compared to ewes fed diets containing fishmeal. In addition, a higher growth rate of lambs and a higher 28-day litter weight was recorded in ewes fed concentrates containing formaldehyde treated soya-bean meal with added rumen protected methionine. There was no significant difference in colostrum quality or in lamb growth rate between ewes fed concentrate containing fishmeal and those fed formaldehyde treated soya-bean meal alone (with no rumen protected methionine). It is apparent from the experiment reported in Chapter 5 and from that of Rodehutscord *et al.* (1999) that formaldehyde treatment may reduce the amount or availability of sulphur containing amino acids and supplements of rumen-protected methionine are necessary to correct this. Arguably the positive effects of protected methionine supplementation would have also been seen if rumen protected methionine was added to diets containing untreated soya-bean meal.

6.3 CONCLUSIONS

The quality of fishmeal protein is dependent on source. Vegetable proteins used in the current series of experiments can be used successfully as alternative protein sources to fishmeal in diets for ewes in late pregnancy and lactation. Formaldehyde treatment of vegetable proteins is successful at reducing rumen degradability and increasing the calculated MP supply. However, this alone did not result in improvements in ewe productivity. It appears that formaldehyde treatment of vegetable protein sources is only really worthwhile if diets for pregnant and lactating ewes are also supplemented with the rumen protected amino acids that are limiting productivity.

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